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TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

Hygiene

HYGIENIC LABORATORY—BULLETIN No. 115
OCTOBER, 1918

I. NOTES ON THE DETECTION OF B. TETANI

By G. W. McCOY and IDA A. BENGTSON

II. THE STANDARDIZATION OF PITUITARY EXTRACTS

By REYNOLD A. SPAETH

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NOTES ON THE DETECTION OF B. TETANI.

By
G. W. McCoy, Director, Hygienic Laboratory,
and
Ida A. Bengston, Sanitary Bacteriologist, United States Public Health Service.

The occurrence of a number of cases of tetanus following vaccination against smallpox, in which the product of a single manufacturer had been used, made it necessary, assuming that the infection was a direct consequence of the vaccination, to endeavor to determine the source of the contamination.

The material for examination consisted of "ivory" points, "sterilized" and unsterilized, glycerinated vaccine virus in bulk, on points, and in capillary tubes; and glycerinated seed vaccine used for propagating the virus.

As the cases of tetanus had all followed the use of "ivory" points, though some of each suspected lot of vaccine had been distributed in capillary tubes, our work was begun by attempting to detect the infection in the points as they reached the manufacturer. It was believed that while the process of sterilization which was employed—steaming in an Arnold sterilizer for an hour on each of two successive days—would destroy tetanus spores if the process were properly carried out, oversight or carelessness on the part of an employee might result in the use of points that had not been steamed or that had been inadequately treated. It was thought, therefore, that the most promising method of approaching the problem was to endeavor to detect infection on the unsterilized uncharged points.

After administrative action which would prevent the further distribution of suspected material and effect the recall of that outstanding had been taken the following experimental work was carried out, though not in the exact order given here.

EXAMINATION OF UNSTERILIZED UNCHARGED "IVORY" POINTS.

The points first tested were planted directly into plain broth fermentation tubes, the broth being made from meat and containing muscle sugar, the tubes having been previously steamed for half an hour to drive out air, for the purpose of bringing about anaerobic conditions in the closed arm. Tests were made by planting a series of tubes with 1 point in each tube, another series with 2, another with 5, and still another with 10. These tubes were not heated after planting, as was the case with the flasks planted later.

The following summarizes the results obtained with points planted in the fermentation tubes:

I. Points obtained by the vaccine manufacturer directly from the manufacturer of "ivory" points. 396 points planted in 78 fermentation tubes—1, 2, 5, or 10 points in each. (Fermentation tubes not heated after planting.)

Tubes planted.	Points in each tube.	Total number of points.
24	1	24
6	2	12
24	5	120
24	10	240
78		396

Growth in closed arm of 77 tubes.[1]

Gas in closed arm of 59 tubes.

Tetanus-like organisms in 41 tubes (but not all examined).[2]

Guinea pigs inoculated, 17 (all negative).

Mice inoculated, 27 (1 positive, 26 negative).

Tetanus symptoms were observed in only one instance. This was in a mouse inoculated with a culture from a tube planted with a single point. The culture produced toxin of such a potency that a mouse injected with 0.0001 c. c. showed slight symptoms in 7 hours, marked symptoms in 22 hours, and died during the second day after inoculation. A mouse protected with 10 units of tetanus antitoxin and injected with the same dose (0.0001 c. c.) of culture showed no symptoms.

Fifteen hundred points from the same firm were distributed equally among 10 Kjeldahl flasks, containing plain broth, previously heated to expel air. Liquid petrolatum was used to overlay the medium in half of the flasks to determine whether this treatment favored the growth of *B. tetani* by improving anaerobic conditions. Three of the flasks were heated after planting to 60° C. for from one-half to one hour, two were heated to 70° C. for one hour, and five were not heated.

One of the flasks heated to 60° C. had been planted with ether-washed points; the purpose of this procedure being to destroy vegetative forms of other organisms, also to dissolve any possible lipoid film which might prevent the broth reaching spores present on the points. Smears made after nine days' incubation and stained by Gram's method, showed tetanus-like organisms in each flask. Tests were made on mice with 1 c. c. of the culture from each flask, and positive results were obtained from two flasks, one of which had been heated to 70° C. for one hour and had not been treated with oil; the other had been planted with the ether-washed points, the flask being treated with oil and heated to 60° C. for half an hour. Heating apparently favored the growth of tetanus organisms in flasks, probably by destroying vegetative forms of other organisms, though some other factor may be concerned.

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[1] In the light of subsequent observations it would have been better to have submitted all of those showing growth in the closed arm to the animal test.

[2] This expression always means organisms resembling *B. tetani* morphologically.

A mouse inoculated with 0.001 c. c. of the culture from one of the flasks showed symptoms of tetanus in 18 hours and died on the third day. A control mouse, inoculated with 0.001 c. c. of the culture and 10 units of tetanus antitoxin, survived.

A guinea pig injected with 1 c. c. of the culture from the second flask, the one which had been planted with the ether-washed points, developed pronounced symptoms of tetanus in 3 days, and died in 5 days. Smaller doses, 1/10, 1/100, and 1/1000 c. c., in mice produced no symptoms, showing that the toxin was not very strong.

Further tests were made by planting one point in each fermentation tube and subjecting the seeded tubes to varying degrees of heat and using different amounts of glucose, varying from 0.1 per cent to 2 per cent. The medium in half the tubes was overlaid with oil.

Fifty-three points were planted in 53 fermentation tubes.

Growth in closed arm of 47; gas in closed arm of 22; tetanus-like organisms in 6; guinea pigs inoculated, 4; mice inoculated, 2.

None of the animals inoculated developed tetanus symptoms.

In view of results obtained later in the inoculation of animals from tubes showing growth in the closed arm it is possible that in this and several later experiments we may have missed one or more positive cultures by failure to inoculate animals from any tubes excepting those which contained tetanus-like organisms.

II. Uncharged points received in an unsterilized condition from several firms manufacturing vaccine virus, whose product was not under suspicion, were tested as follows:

Manufacturer A.—One thousand two hundred points planted in 4 Kjeldahl flasks—300 each (heated to 65° C. for 40 minutes). Tetanus-like organisms, 4; mice inoculated, 4; tetanus symptoms in 1 (dose of 1 c. c. produced symptoms in 24 hours).

Manufacturer B.—Four thousand five hundred points planted in 15 Kjeldahl flasks (9 heated to 65° C. for 40 minutes; 6 heated to 70° C. for 1 hour). Tetanus-like organisms in 5 (not all examined); mice inoculated, 25.

One c. c. from each flask and from some flasks smaller doses were also employed. No tetanus symptoms developed in mice inoculated from these flasks.

Manufacturer C.—Three thousand three hundred points planted in 11 Kjeldahl flasks (heated to 65° C. for 40 minutes). Tetanus-like organisms in 11; mica inoculated, 16 (two mica given different doses from 5 flasks).

Tetanus symptoms developed in mice inoculated with cultures from three different flasks. One inoculated with 0.1 c. c. showed symptoms in 22 hours and two others inoculated with 1 c. c. showed symptoms in 18 hours and 2 days, respectively.

EXAMINATION OF "STERILIZED" IVORY POINTS.

A large number of ivory points, "sterilized" and ready for charging with virus were secured from the manufacturer whose product was under suspicion and were examined, with the following results:

Manufacturer D.—Eight hundred and seventy points planted in 168 plain broth fermentation tubes—1 to 10 in each. (Fermentation tubes not heated after planting.[1]) Growth in closed arm of 104; gas in closed arm of 30; tetanus-like organisms in 23; guinea pigs inoculated, 6; mice inoculated, 32.

No tetanus symptoms developed in any of these animals.

Tests were then made by planting each of 20 Kjeldahl flasks with 200 points from five different packages, four flasks being planted with points from each cloth-wrapped package. Two flasks of each set were covered with petrolatum and 2 left without it. All were heated to 65° C. for 40 minutes and then incubated for 10 days. Smears showed tetanus-like organisms from all flasks.

Animals were inoculated with 1 c. c. and 0.1 c. c. of culture from each flask. Tetanus symptoms developed in animals injected with the growth from 4 flasks planted with points from 3 different packages, indicating that at least 3 out of the 5 supposedly sterilized packages contained living *B. tenani* spores.

One c. c. of the culture from each of the four positive flasks produced symptoms and death of mice within 24 hours, except in one case, in which the mouse died on the second day, and 0.1 c. c. also gave symptoms in three cases within 24 hours and death in 24 to 48 hours.

Several tests were made to determine whether a temperature of 100° C. in the Arnold sterilizer would kill tetanus spores on ivory points artificially contaminated.

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[1] Wherever tubes or flasks are described as "not heated" or "unheated," reference is made to seeded tubes or flasks which have not been subjected to a temperature above 37° C. after planting, though, of course, it is understood that all tubes and flasks were heated to 100° C. for 30 minutes to expel air before planting.

The points were treated in a Petri dish with a heavy suspension of tetanus spores obtained from a culture isolated from court plaster and allowed to dry at incubator temperature. Six points thus contaminated were then placed in the interior of a tightly wrapped cloth package of points (approximately 1.000) and heated for one hour in the Arnold sterilizer. They were then planted in plain broth fermentation tubes, but none showed growth. Control points contaminated in the same way, but unheated, showed growth characteristic of the tetanus organism. It is, of course, possible that under varying conditions of cultivation and treatment the resistance of tetanus spores to heat may vary.

WASHINGS FROM "STERILIZED" UNCHARGED POINTS.

In these tests four cloth-wrapped packages of "sterilized" points obtained from manufacturer D were used. The points were distributed in 100 c. c. bottles containing sterile water, using 150 to 200 points in each bottle. The bottles were shaken vigorously for five minutes, the fluid poured into sterile centrifuge tubes and centrifuged for half an hour. The supernatant fluid was poured off, leaving about 5 c. c. of sediment suspension in each tube. This was distributed in broth fermentation tubes so that the washings from each 150 to 200 points were planted in 10 tubes, a total of approximately 4,400 points being used. After planting, the fermentation tubes were heated for half an hour at 65° C.

Number of fermentation tubes planted, 239; growth in closed arm, 226; gas in closed arm, 141; tetanus-like organism in 47 (not all examined); guinea pigs injected, 23.

Pooled specimens from several tubes were injected into guinea pigs, each guinea pig receiving 2 c. c. of the pooled specimen, about 0.2 c. c. from each tube. None of the guinea pigs developed tetanus symptoms.

On account of shortage of animals it was not possible to test as many of the cultures for toxin as was desirable.

EXAMINATION OF CHARGED "IVORY" POINTS.

The material used in this test was from lots that came under suspicion by reason of association with one or more cases of tetanus, or from lots that were prepared at about the same time.

At first the technique for the detection of tetanus organisms on charged points consisted in removing, with a pair of forceps under aseptic conditions, the ivory point from the capsule containing the one or two drops of virus, dipping the point into the broth fermentation tube and allowing it to remain for 30 to 60 seconds, in order to give time for the adhering virus to diffuse into the broth. The virus remaining in the capsule was then removed with a capillary pipette and the inside of the capsule washed with a few drops of broth transferred from the tube to the capsule with the same pipette; this was also planted in the fermentation tube, insuring as far as possible the transfer of the whole content of the capsule to the broth tube.

Tests were made with charged points selected from 14 different laboratory numbers, representing as many lots of vaccine virus, the number of points from each lot varying from 1 to 40. Tubes were not subjected to heating after planting.

Vaccine virus from 237 charged points planted in 237 fermentation tubes. Growth in closed arm of 56, gas in closed arm of 6, tetanus-like organisms in 2.

Mice inoculated with 1 c. c. of cultures from the two tubes showing suspicious organisms remained well.

Many of the tubes planted by this method were sterile.

The few tubes developing gas and showing suspicious organisms in smears contrast with the much larger number of tubes planted with points sterilized and unsterilized which showed gas and tetanus-like organisms.

In the remainder of the work with charged points, the end of the ivory point which was immersed in the vaccine virus in the capsule was cut off and planted in plain broth, without attempting to remove the virus remaining in the capsule. Sterile scissors were used in this procedure, and the points were collected in a sterile conical glass and transferred from this to fermentation tubes or Kjeldahl flasks. In these tests all the tubes and Kjedahl flasks were heated to 65° C. for 40 minutes. For the most part, tests were made in the Kjeldahl flasks, 100 to 300 cut-off points being planted in each flask, but in a few cases, where the number was considered too small, fermentation tubes were used.

Seventy-seven points (from 4 laboratory numbers) were planted in 18 fermentation tubes. Growth in the closed arm and gas, with tetanus-like organisms, were present in one tube, but the mouse test on this tube was negative.

Five thousand three hundred points (from four laboratory numbers) planted in 23 Kjeldahl flasks (heated to 65° C. for 40 minutes after planting). Tetanus-like organisms in 16; mice inoculated (0.1 c. c. to 1.0 c. c.), 30.

Tetanus symptoms developed in the mice inoculated from 4 of the 23 flasks, all of these having been planted from samples bearing the same laboratory number. A dose of 0.25 c. c. in one case produced symptoms in 18 hours and death within 24 hours. A mouse given the same amount of culture and 10 units of tetanus antitoxin survived. In another case tetanus symptoms were obtained in 3 days with a dose of 1 c. c., and in another within 24 hours on a dose of 1 c. c. The fourth mouse died within 24 hours on a dose of 0.1 c. c.

EXAMINATION OF FILLED CAPILLARY TUBES.

While the epidemiological evidence did not point to the presence of infection in capillary tubes, it was considered desirable to examine a large number. As the empty capillary tubes were sterilized by steam under pressure or by the use of a very high temperature in the dry-air sterilizer, it was believed that infection from this source was unlikely. If infection of filled tubes had been found, we should have considered the glycerinated lymph the probable source rather than the ivory points which our work had brought under suspicion.

The tests on vaccine virus in capillary tubes were made by planting the contents in fermentation tubes, each fermentation tube being planted with vaccine from 1 to 10 capillary tubes. Some of these were not heated and some were heated to 65° C. for 40 minutes.

Manufacturer D.—One thousand four hundred and twenty-five capillary tubes planted in 219 fermentation tubes. Growth in closed arm, 9; gas in closed arm, 2; tetanus-like organisms in 0; mice inoculated, 2.

No tetanus symptoms were produced in either mouse inoculated with material from the tubes showing gas.

No growth in either the open or closed arm was present in 169 tubes, and 41 showed growth only in the open arm.

EXAMINATION OF GLYCERINATED LYMPH.

Rather large amounts of bulk lymph from various lots under suspicion were examined, all with negative results. These included large quantities from the lots that had been used to charge points that had given positive results in the tests that have been reported in the section on "Examination of charged 'ivory points,'" pages 10–12.

Seed and bulk vaccine were tested by planting 1, 5, and 20 drop amounts in plain broth fermentation tubes, and also by planting in Kjeldahl flasks. The following is a statement of seed vaccine planted:

Seed vaccine.—Sixteen samples in 120 fermentation tubes (not heated after planting): Growth in closed arm of 22; gas in closed arm of 4; tetanus-like organisms, 0.

Fifteen mice were inoculated, none of which showed symptoms. In some cases pooled specimens from several tubes were used for the inoculation.

In 7 out of the 15 samples no growth was obtained in either the closed or open arm. Gram positive cocci or very short gram positive rods were the usual contamination when growth occurred.

Some of the fermentation tubes were heated to 60° C. for half an hour after planting; others were not heated.

Bulk vaccine.—Sixteen samples representing approximately 75 c. c. or 2,250 vaccinations in 180 fermentation tubes. Growth in closed arm of 45; gas in closed arm of 30 (many showed large amounts of gas and were found to contain *B. aerogenes capsulatus*); mice injected, 5 (used pooled specimens from several tubes in injections); tetanus symptoms in none. Five samples were sterile.

Seventeen samples representing 250 c. c. or 7,500 vaccinations planted in 31 Kjeldahl flasks. Tetanus-like organisms in 1; mice injected, 40 (1 or more from each flask). No tetanus symptoms developed in any of the mice inoculated.

Some of the samples were heated to a temperature of 65° C. for one hour before planting, and some were heated to the same temperature for one hour after having been planted.

From this portion of our work we drew the conclusion that *B. tetani* is a relatively common contamination of the ivory points as shipped to vaccine producers; that it may be present on points after charging with vaccine;[1] and that vaccine virus is unlikely to be contaminated with the organism under consideration.

TECHNIQUE.

In carrying out the experimental work just considered and that to follow we have freely consulted and derived much help from the paper of Francis.[2]

Media employed.—In the early part of the investigation a few glucose broth tubes were used in some of the experiments, but in the greater part of the work the medium consisted of plain broth made from fresh lean beef, containing muscle sugar, strained before heating, and sterilized in streaming steam, neutral to phenolphthalein, tubed in Smith fermentation tubes, or contained in 500 c. c. Kjeldahl flasks. This was in accordance with the results obtained by Francis, who found that glucose broth without heating after seeding was unsuitable for the detection of tetanus spores in vaccine virus on account of high acid production, due to the rapid development of other organisms which by their action on glucose produced a high acidity.

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[1] The sale of vaccine virus on or with points in interstate traffic has been prohibited by an order of the Secretary of the Treasury.

[2] Laboratory Studies of Tetanus, by Edward Francis. Hygienic Laboratory Bulletin No. 95, 1914.

Heating to destroy contaminations.—As we believed that we were dealing with a very scanty contamination of *B. tetani*, it was considered unwise to heat material to 80° C. for an hour preliminary to incubation as is usually recommended for the purpose of destroying other vegetative forms. Hence in the majority of the tests we heated the broth after seeding to 65° C. for from 40 minutes to 1 hour.

Anaerobiosis.—We used freshly steamed Smith fermentation tubes quite extensively, tipping immediately after heating to expel the resulting bubble from the closed arm. When large amounts of material were to be dealt with, 500 c. c. Kjeldahl flasks were found very satisfactory. They were used just after they had been sterilized or were freshly steamed for one hour. Liquid petrolatum was placed over the fluid in the case of most of the flasks, though a few were incubated without the oil covering. The comparative results of this procedure are shown in the following table:

Flasks.	Positive tetanus.
79 were treated with petrolatum after planting	12
19 without petrolatum	2
98	14

The difference in results by the two methods is not sufficiently marked to justify any conclusion as to the superiority of either.

Nature and amount of material planted.—This varied from a single ivory point planted in a fermentation tube to 300 points planted in the Kjeldahl flasks. The details are tabulated here:

Unsterilized uncharged points:			
Fermentation tubes	90	points (1 in each tube)	1 positive.
	16	points (2 in each tube)	0 positive.
	120	points (5 in each tube)	0 positive.
	240	points (10 in each tube)	0 positive.
Flasks	10,500	points in 40 flasks (150 to 300 in each flask)	6 positives.
Total	10,966		
Sterilized uncharged points:			
Fermentation tubes	168	points (1 in each tube)	0 positive.
Flasks	4,000	points in 20 flasks	4 positives.
Washings from	4,400	points in 239 fermentation tubes	0 positive.
Total	8,568		
Cut-off charged points:			
Fermentation tubes	77	points in 18 fermentation tubes (1 to 6 points in each tube)	0 positive.
Flasks	5,300	in 23 flasks (100 to 300 in each flask)	4 positives.

Charged points (vaccine only planted):		
Fermentation tubes	237 (1 in each)	0 positive.
Capillary tubes:		
Fermentation tubes	87 capillary tubes (1 in each)	0 positive.
	1,340 capillary tubes (10 in each)	0 positive.
Seed vaccine:		
Fermentation tubes	16 samples	0 positive.
Bulk vaccine:		
Fermentation tubes	16 samples (2,250 vaccinations)	0 positive.
Kjeldahl flasks	17 samples (7,500 vaccinations)	0 positive.

MICROSCOPIC EXAMINATION OF CULTURES.

In the early part of the work microscopic examinations were made of smears from nearly all fermentation tubes which showed growth and gas in the closed arm and from all the flasks. Tests were made on animals when the smears showed tetanus-like organisms. However, the results checked so poorly that in the latter part of the work not much importance was attached to the appearance of smears and in some cases they were omitted.

B. tetani in pure culture has a very distinctive appearance. It is usually a gram positive rod in the first 24 hours. Spores then begin to develop and appear in the early stages as gram positive, solidly stained, terminal, or slightly subterminal, bodies, rounded or lanceolate in form. In three to four days typical tetanus organisms, with large, usually gram positive, terminal round spores are present. The rods soon lose the property of retaining the gram stain, and in old cultures the spore also apparently loses the power to hold the stain, so that the organism is not easily distinguished from certain other terminal-spored rods. Our experience clearly indicates that a smear is not a reliable index of the presence or absence of *B. tetani*, as determined by the animal test. Organisms with rounded terminal spores, many times indistinguishable morphologically from the *B. tetani* controls, were present, almost without exception, in all of the tubes and flasks planted with the ivory points, although few showed the presence of tetanus toxin by animal tests. Vaccine virus, on the other hand, with a few exceptions, failed to show any terminal-spored organisms.

Production of gas by B. tetani.—There seems to be some uncertainty as to the production by the tetanus bacillus of gas in plain broth made as described above. Our own experience has been that gas was uniformly produced by the pure cultures of *B. tetani* we have worked with, and usually in the presence of other organisms. On the other hand, gas was produced many times in tubes planted with "ivory points" where tetanus spores were absent.

Odor of cultures of B. tetani.—The characteristic garlic-like odor of tetanus cultures was present in all of the cultures that proved positive by the animal test; but it was also present in many cultures that proved negative; indeed, the large majority of the flasks gave this odor.

Period of growth of cultures prior to animal tests.—Animal tests were made after an incubation period varying from 5 to 18 days in the case of fermentation tubes; the majority, however, after a period of about 10 days. All of the cultures which were tested proved to be negative with one exception, and this one was incubated for a period of 8 days. Tests with the same culture from the same fermentation tube were made on mice after 15 days' growth, a dose of 0.0001 c. c. proving to be lethal after this length of time.

The flasks were incubated as a rule from 9 to 11 days. Of the 14 positive flasks, 3 were incubated 9 days, 9 were incubated 10 days, and 2 were incubated 11 days.

Apparently incubation at 37° C. in plain broth for 10 days is favorable for the development of tetanus toxin in mixed cultures in which many other species of organisms are present.

Tests of cultures on animals.—The final decision as to the presence of the tetanus organism was based on the development of characteristic symptoms of tetanus in laboratory animals. It would be more satisfactory to isolate *B. tetani* in pure culture and use this for all tests; but, save in exceptional cases, the time consumed in this process does not warrant the attempt, since the symptoms produced by tetanus toxin in guinea pigs and mice are quite characteristic and confirmation can readily be secured if desired, as was done in this work in several instances by the inoculation of animals in duplicate, protecting one by means of a simultaneous inoculation of tetanus antitoxin, while the other serves as a control.

Incubation period.—Most of the tests were carried out on mice; only a few guinea pigs were used. Symptoms appeared in mice, for the most part, within 12 hours after inoculation, and when overwhelming doses were used death often occurred in less than 18 hours without characteristic manifestations. In only a few cases were symptoms observed after a longer period than two or three days, being recorded as occurring in 7, 8, 10, 14, 18, and 24 hours, and occasionally in from 2 to 3 days. The one guinea pig that was positive showed symptoms in two days. Suspicious symptoms often developed in mice the first day after inoculation and became marked on the following day.

In general, as noted, the appearance of symptoms was very prompt. Only in cases where both culture and antitoxin were administered and the amount of antitoxin was too small to neutralize the toxin were symptoms delayed for three or four days, and in one case as long as eight days.

Symptoms.—All of the inoculations into animals were made into the posterior part of the body. In guinea pigs the earliest symptoms are stiffness of the body and lameness of the hind legs. The animal soon becomes unable to get on its feet when laid on its back.

In mice the body becomes curved; this and loss of use of the hind legs and general spasticity are outstanding symptoms. Later, the animal becomes unable to move about; the body is strongly flexed laterally (pronounced pleurothotonos) and remains so even after death. Occasionally an animal will recover, having received a sublethal dose.

Filtration of cultures.—In carrying out the animal tests, most of the inoculations were made with unfiltered cultures, and in the large majority of cases, typical tetanus symptoms were obtained when suitable doses were used. Animals sometimes died without typical manifestations of tetanus after inoculation with unfiltered cultures, while animals given smaller doses of the same unfiltered cultures showed typical symptoms, indicating that the indefinite symptoms were due rather to too large a dose of toxin than to the presence of contaminating organisms in the material injected. In a very few cases when doubtful symptoms were produced on inoculation with cultures, typical tetanus was obtained with the filtrate.

EXPERIMENTAL WORK.

Experimental work was carried out to determine, as far as possible, what conditions are most favorable for the growth of *B. tetani* and the development of tetanus toxin, particularly in relation to possibly contaminated vaccine virus. The greater part of this work followed that considered in the preceding section (pp. 3–20).

For this purpose a number of tests were made with suspensions of spores of *B. tetani* alone, with the spores in association with other organisms, and with vaccine virus artificially contaminated with spores. Both cultural tests and direct inoculation experiments on animals were made.

Three different cultures of the organism were used, including:

A suspension of spores of *B. tetani* in broth prepared by Dr. Francis (Hygienic Laboratory Bulletin 95) and kept in sealed glass bulbs for a period of about five years at a temperature of 15° C. designated A; a culture of the organism isolated from court plaster (McCoy, Leake, and Corbitt, U. S. Public Health Service, Public Health Reports, vol. 32, pp. 1450–1454, Sept. 7, 1917), designated B; and a culture isolated from a plain broth fermentation tube, planted with an unsterilized ivory point, designated C (p. 5). In order to avoid as far as possible the presence of viable vegetative forms, broth suspensions of cultures B and C, which were about a month old, and the original suspension of culture A referred to above, were used.

In carrying out the various tests, the medium was always heated to 100° C. for one-half hour before planting and tipped to expel air. In each experiment tubes containing broth of the same lot were used in order to avoid as far as possible discrepancies which might arise by using different lots. The medium used, as in the previous section (p. 15), was plain broth, neutral to phenolphthalein.

In carrying out tests, especially those with artificially contaminated vaccine virus, it was the purpose to use relatively small amounts of contamination, in order, as far as possible, to obtain results that would be of practical value in the detection of *B. tetani* spores in minimal amounts, as they might possibly occur in vaccine under manufacturing conditions, and also in order that erroneous interpretations might not be placed on tests made on material containing excessive amounts of *B. tetani* organisms. It is probably true that conditions which are unfavorable for the development of very few organisms would, to a certain extent, be overcome in the presence of a large number.

Preliminary "titrations," to determine the limit of fairly constant growth, were therefore made of the three cultures which were used throughout the work. The following tables show the results of these determinations.

In all cases the various dilutions of spore suspensions were planted in Smith fermentation tubes containing about 25 c. c. of broth. Dilutions of the spore suspensions were made with sterile 0.85 per cent salt solution.

B. TETANI A.

Dilution of spore suspension.	Not heated.						Heated to 65° C. (1 hour).					
1/100	g^2	g^1	g^1	g^2	......	......	g^2	g^2	g^1	g^3	......	......
1/1,000	g^1	g^1	g^1	g^2	......	......	g^3	g^3	g^2	g^2	......	......
1/10,000	g^3	g^2	g^3	g^3	g^2	g^3	g^2	g^2	g^2	g^2	g^2	g^2
1/100,000	g^3	0	g^4	g^4	g^2	g^2	g^3	g^4	g^2	g^2	g^3	g^2
1/1,000,000	0	g^3	g^3	0	0	g^3	0	0	g^3	g^2	0	g^2
1/10,000,000	0	0	0	0	0	0	0	0	0	0	0	0

B. TETANI B.

Dilution of spore suspension.	Not heated.						Heated to 70° C. (1 hour).					
1/5,000,000	g^2	g^6	g^2	g^2	g^4	g^2	g^3	g^2	g^3	g^2	g^2	g^3
1/15,000,000	0	g^4	g^4	g^4	g^4	g^{11}	g^7	g^3	g^2	g^2	g^2	g^2
1/25,000,000	g^9	g^5	g^7	0	g^8	0	g^3	g^4	g^2	g^3	g^6	g^3
1/50,000,000	(1)	(1)	(1)	(1)	(1)	(1)	g^2	g^2	g^2	g^2	0	g^3
1/100,000,000	(1)	(1)	(1)	(1)	(1)	(1)	0	g^2	g^2	0	g^2	0
1/200,000,000	(1)	(1)	(1)	(1)	(1)	(1)	g^2	g^2	g^2	g^2	g^2	0
1/400,000,000	(1)	(1)	(1)	(1)	(1)	(1)	g^2	0	0	g^2	0	0
1/800,000,000	(1)	(1)	(1)	(1)	(1)	(1)	0	g^2	g^2	g^2	0	0

B. TETANI C.

Dilution of spore suspension.	Not heated.					Heated to 70° C. (1 hour).				
1/100,000	g^2	g^2	g^2	0	0	g^2	g^2	g^2	g^2	g^2
1/500,000	0	0	g^3	0	g^2	g^2	g^2	g^2	g^2	g^3
1/1,000,000	0	g^3	0	g^3	g^2	g^2	g^2	0	g^2	g^2
1/2,000,000	g^2	0	0	g^3	0	0	g^2	0	g^2	g^3
1/4,000,000	0	0	0	0	0	0	g^2	0	g^2	g^2

g=gas (slight growth in the closed arm usually appeared 6 to 24 hours before gas was observed).
Number refers to day on which gas appeared.
0=no growth.
1—Not planted.

B. tetani A showed fairly constant growth in a dilution of 1/100,000, B in 1/25,000,000 and 1/50,000,000, and C in 1/100,000 to 1/500,000, and tests to detect the presence of the organism were carried out for the most part with dilutions not exceeding these limits.

EFFECT OF VARIOUS TEMPERATURES ON THE DEVELOPMENT OF SPORES OF B. TETANI.

A number of tests were carried out, with the three cultures used in this work, to determine more accurately the effect of heating to 80° C., which is generally considered to be a temperature sufficiently high to destroy vegetative forms of organisms, which might interfere with the detection of *B. tetani* while still allowing spores of the latter to survive. It was suspected that a temperature of 80° C. might be excessive in the case of high dilutions, since it was thought probable that some spores of *B. tetani* are destroyed at this temperature, and a minimum of spores necessary for growth might not survive. This suspicion was justified to some extent by the experimental work.

A control set of tubes, unheated, was always used in these tests. The characteristic growth of *B. tetani* starting in the closed arm, and gas production, were considered as positive in recording the results, since no other organisms were present in the cultures used.

B. TETANI A.

Dilution of spore suspension.	Not heated.						Heated to 65° C. (1 hour).						Heated to 80° C. (1 hour).					
1/100	g^2	g^1	g^1	g^2	....	....	g^2	g^2	g^1	g^3	....	....	g^2	g^2	g^1	g^1	....	....
1/1,000	g^1	g^1	g^1	g^2	....	....	g^3	g^3	g^2	g^2	....	....	g^2	g^2	g^2	g^2	....	....
1/10,000	g^3	g^2	g^3	g^3	g^2	g^3	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2
1/100,000	g^3	0	g^4	g^4	g^2	g^2	g^3	g^4	g^2	g^2	g^3	g^2	g^2	g^2	g^2	g^2	g^2	g^2
1/1,000,000	0	g^3	g^3	0	0	g^3	0	0	g^3	g^2	0	g^2	g^3	g^3	g^3	g^3	g^2	0
1/10,000,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	[a]	g^4	....
Results from all dilutions up to and including 1/1,000,000	22 positive, 4 negative.						23 positive, 3 negative.						25 positive, 1 negative.					

[a] Not included in summary.

This experiment conducted with a suspension of spores which had stood several years showed, if anything, that preliminary heating to

80° C. was more favorable than heating to a lower temperature. In the light of later work, we were inclined to believe that the hardier spores along had survived in the suspension.

Organism B, a more recently isolated one, was tested at several intermediate temperatures, in addition to 80° C. and not heated.

B. TETANI A.

Dilution of spore suspension.	Not heated.						Heated to 65° C. (1 hour).						Heated to 70° C. (1 hour).					
1/100,000	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^6	g^4	g^5	g^4	g^2	g^2	g^2	g^3	g^5	g^3	g^4
1/1,000,000	g^2	g^2	g^2	g^3	g^2	g^2	g^2	g^2	g^3	g^3	g^3	g^3	g^2	g^3	g^3	g^3	g^2	g^2
1/10,000,000	g^5	g^5	g^5	g^3	g^4	g^7	g^3	g^3	g^3	0	g^5	g^6	g^3	g^3	g^3	g^3	g^3	g^2
	18 positive, 0 negative.						17 positive, 1 negative.						18 positive, 0 negative.					

Dilution of spore suspension.	Heated to 75° C. (1 hour).						Heated to 80° C. (1 hour).						Heated to 100° C. (1 hour).
1/100,000	g^2	g^2	g^2	g^4	g^2	g^4	g^2	g^4	g^2	g^5	g^5	g^5	All negatives 7 days.
1/1,000,000	g^5	g^3	g^4	g^2	g^3	g^2	g^2	g^4	g^5	g^5	g^7	g^3	Do.
1/10,000,000	g^3	g^5	g^3	g^3	g^3	g^{13}	g^4	g^3	g^6	0	g^5	g^5	Do.
	18 positive, 0 negative.						17 positive, 1 negative.						0 positive, 18 negative.

In this test a temperature of 70° C. appeared somewhat more favorable than 80° C., in that growth was more prompt in the tubes heated to 70° C. than in those heated to 80° C., though finally there was a difference of only 1 tube more in favor of the 70° C. temperature. A temperature of 100° C. for one hour killed all organisms.

The test was continued with a higher dilution, as shown in the following table:

B TETANI B.

Dilution of spore suspension.	Not heated.						Heated to 60° C. (1 hour).					
1/5,000,000	g^2	g^6	g^2	g^2	g^4	g^2	g^7	g^4	g^4	g^5	g^7	g^6
1/15,000,000	0	g^4	g^4	g^4	g^4	g^{11}	g^3	....	g^4	g^5	g^3	g^7
1/25,000,000	g^9	g^5	g^7	0	g^8	0	g^3	g^3	g^3	g^{16}	g^7	g^5
	15 positive. 3 negative.						17 positive, 0 negative.					

Dilution of spore suspension.	Heated to 70° C. (1 hour).						Heated to 80° C. (1 hour).					
1/5,000,000	g^3	g^2	g^3	g^2	g^2	g^2	g^5	g^5	g^4	g^2	g^2	g^1
1/15,000,000	g^7	g^3	g^2	g^2	g^2	g^2	g^4	g^7	0	g^2	g^2	g^2
1/25,000,000	g^3	g^4	g^7	g^3	g^6	g^2	0	g^4	g^6	g^2	g^6	0
	18 positive, 0 negative.						15 positive, 3 negative.					

In this test also a temperature of 70° C. seemed more favorable than 80° C., and it also appeared that better results were obtained by heating to 60° C. and to 70° C. than by not heating, but, as was the case in the preceding experiments, the difference was not striking.

Organism C, isolated from uncharged, unsterilized "ivory" points, was tested in the same way, with the following results:

B TETANI C.

Dilution of spore suspension.	Not heated.					Heated to 70° C. (1 hour).					Heated to 80° C. (1 hour).				
1/100,000	g^2	g^2	g^2	0	0	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^{12}	g^3
1/500,000	0	0	g^3	0	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^3	g^2	g^2	g^2
1/1,000,000	0	g^3	0	g^3	g^2	g^2	g^2	0	g^2	g^2	0	0	0	0	0
1/2,000,000	g^2	0	0	g^3	0	0	g^2	0	g^2	g^3	0	0	0	g^2	0
1/4,000,000	0	0	0	0	0	0	g^2	0	g^2	g^2	0	0	0	0	0
	10 positive, 15 negative.					20 positive, 5 negative.					11 positive, 14 negative.				

In this test the favorable results obtained by heating to 70° C, in contrast to heating to 80° C. are more striking than in the preceding tests.

Summary of results obtained by subjecting broth tubes inoculated with pure cultures of B. tetani in high dilutions to various temperatures.

	Unheated.		Heated to 60° C. (1 hour).		Heated to 65° C. (1 hour).		Heated to 70° C. (1 hour).	
	Positive.	Negative.	Positive.	Negative.	Positive.	Negative.	Positive.	Negative.
B. tetani A.: Dilutions 1/100,000 to 1/1,000,000	8	4	....	....	9	3	....	....
B. tetani B.:								
Dilutions 1/100,000 to 1/10,000,000	18	0	....	....	17	1	18	0
Dilutions 1/5,000,000 to 1/25,000,000	15	3	17	0	....	....	18	0
B. tetani C.: Dilutions 1/100,000 to 1/4,000,000	10	15	....	....	....	....	20	5
Total	51	22	17	0	26	4	56	5
Per cent	70	30	100	....	87	13	92	8

	Heated to 75° C. (1 hour).		Heated to 80° C. (1 hour).		Heated to 100° C. (1 hour).	
	Positive.	Negative.	Positive.	Negative.	Positive.	Negative.
B. tetani A.: Dilutions 1/100,000 to 1/1,000,000	....	....	11	1	....	....
B. tetani B.:						
Dilutions 1/100,000 to 1/10,000,000	18	0	17	1	0	18
Dilutions 1/5.000,000 to 1/25,000,000	....	....	15	3	....	....
B. tetani C.: Dilutions 1/100,000 to 1/4,000,000	....	....	11	14	....	....
Total	18	0	54	19	0	18
Per cent	100	....	74	26	....	100

Three tests were made to determine whether a temperature of 80° C. tended to inhibit the development of tetanus spores in deep agar shake cultures. Parallel tests were run at 70° C. and also with no heating. Readings were made after three days' incubation.

B. TETANI C.

Dilution of spore suspension.	Unheated.	Heated to 70° C. (1 hour).	Heated to 80° C. (1 hour).
1/100,000	Numerous colonies.	Numerous colonies.	Few colonies.
1/250,000	do	do	0
1/500,000	do	0	0
1/1,000,000	do	Numerous colonies.	0
1/2,000,000	0	0	0

A test was then made with the same culture with a control of the same dilutions planted in fermentation tubes, the agar stabs and fermentation tubes being heated in the Arnold sterilizer to the desired temperature at the same time.

B. TETANI C.

Dilution of spore suspension.	Unheated.						Heated to 70°. (1 hour).						Heated to 80° C. (1 hour).					
	Agar shake culture.	Fermentation tubes.					Agar shake culture.	Fermentation tubes.					Agar shake culture.	Fermentation tubes.				
1/100,000	Numerous colonies.	g^2	g^2	g^2	0	0	Numerous colonies.	g^2	g^2	g^2	g^2	g^2	0	g^2	g^2	g^2	g^2	g^2
1/500,000	do	0	0	g^3	0	g^2	20 colonies	g^2	g^2	g^2	g^2	g^3	0	g^2	g^3	g^2	g^2	g^2
1/1,000,000	Few colonies	0	g^3	0	g^3	g^2	6 colonies	g^2	g^2	0	g^2	g^2	0	0	0	0	0	0
1/2,000,000	Numerous colonies.	g^2	0	0	g^3	0	3 colonies	0	g^2	0	g^2	g^3	0	0	0	0	g^2	0
1/4,000,000	Few colonies.	0	0	0	0	0	0	0	g^2	0	g^2	g^2	0	0	0	0	0	0

A test was also made in deep agar tubes with *B. tetani* A spores, which had been kept in a cold room at 15° C. for several years.

	Not heated.	Heated to 70° C. (1 hour).	Heated to 80° C. (1 hour).
1/10,000	16 colonies	Many colonies	10 colonies.
1/100,000	Many colonies	20 colonies	0
1/500,000	8 colonies	Many colonies	2 colonies.
1/1,000,000	10 colonies	3 colonies	0

A temperature of 70° C. appeared more favorable than 80° C. both by the tests carried out in fermentation tubes and in deep agar shake cultures, and accordingly throughout most of the remainder of the work this temperature was used for heating.

The comparative results obtained in a number of tests of heating pure cultures to 70° C. for one hour and not heating are shown in the following:

B. TETANI A.

Dilution of spore suspension.	Positive.	Negative.
1/100,000:		
Unheated	11	1
Heated to 70° C	12	0

B. TETANI B.

1/25,000,000:		
Unheated	8	4
Heated to 70° C	10	1
1/50,000,000:		
Unheated	2	7
Heated to 70° C	8	1
Total—		
Unheated	10	11
Heated to 70° C	18	2

B. TETANI C.

1/100,000:		
Unheated	6	0
Heated to 70° C	6	0
1/500,000:		
Unheated	16	2
Heated to 70° C	18	0
1/1,000,000		
Unheated	6	6
Heated to 70° C	10	2
Total—		
Unheated	28	8
Heated to 70° C	34	2

Summary of three cultures (unheated and heated to 70° C. for 1 hour).

	Positive.	Negative.
Unheated	87 (68 per cent)	39 (32 per cent).
Heated to 70° C. (1 hour)	116 (94 per cent)	8 (6 per cent).

The tests, together with those included in heating tests previously described, indicate that heating to a temperature of 70° C. after planting was more favorable in the case of organisms B and C to the growth of *B. tetani* in pure culture in high dilutions than not heating. We have no satisfactory explanation for this apparent anomaly.

Effect of vaccine virus on the development of tetanus toxin.

Tests were made to determine to what extent the presence of ordinary bacterial contaminations of the vaccine or the vaccine itself affects the probabilities of detecting *B. tetani* with the use of plain broth as a culture medium.

In making tests with mixtures of vaccine virus and spores of *B. tetani* and also in the case of other organisms in association with *B. tetani*, parallel sets of tubes inoculated with the same amounts of the spores in pure culture as were used in the mixture were always run. In this way, by a comparison of the two, an estimate of the inhibitory or the favorable effect of the vaccine virus or of the other organisms on the growth of the bacillus could be formed. In other words, this control showed just what might be expected of the tetanus organism alone under the conditions of each experiment.

A test was made with a 1/5,000,000 suspension of spores of tetanus culture B and a pooled specimen of several different samples of seed vaccines (A-G) ground about one year previously and probably, therefore, containing few organisms. One cubic centimeter of the suspension of tetanus spores and 0.5 c. c. of vaccine were used throughout. Controls of the vaccine alone were also run. None of the tubes was heated.

B. TETANI B.

Spores+vaccine mixture.						Spores.					
g^2 g^2 g^2	g^3 g^2 g^{2}_{T}	g^2 g^3 g^3	g^5 g^2 g^3	g^2 g^4 g^3	g^2 g^3 g^2	g^2 g^2 g^3	g^3 g^2 g^2	g^3 g^2 g^2	g^3 g^2 g^3	g^2 g^3 g^2	g^3 g^3 g^2

T indicates positive tetanus symptoms in mice.

In this test, apparently, the presence of vaccine in 0.5 c. c. amounts had no inhibitory effect on the growth of *B. tetani*, judging from the appearance of the growth and the presence of gas, though not a sufficient number of animal tests were made to determine this definitely. It is probable that the number of spores of *B. tetani* was too high to determine the effect of vaccine on relatively small numbers of the tetanus organism.

Another test was made with a 1/1,000,000 dilution of *B. tetani* spores A (a relatively high dilution this spore suspension) and a mixture of a pooled specimen of three vaccine viruses (H–J) ground between 6 and 10 months prior to use in the test, using the same amounts as above, and heating to 65° C. for one hour.

B. TETANI A.

1/1,000,000 dilution spore suspension and vaccine mixture.							1/1,000,000 dilution of spore suspension.					
0 g^{4}_{T} 0	0 0 g^3	0 0	0 0 g^{4}_{T}	0 0 0	.... 0 g^{2}_{T}	g^{4}_{T} 0	g^3 g^4 g^2	g^5 0 g^{5}_{T}	g^6 g^4 g^5	g^4 g^4 g^5	g^3 g^4 g^{4}_{T}	g^4 g^4 g^4
5 positive. 13 negative.							18 positive. 1 negative.					

NOTE.—0 indicates no growth in closed arm, though growth may have been present in the open arm of tubes planted with the mixture of spores and vaccine.

It appears that in this test in which a high dilution of spore suspension was used, and in which the tubes were heated, the growth of *B. tetani* was inhibited.

Further comparative tests were made to determine what effect vaccine virus had on the growth of *B. tetani* in fermentation tubes under different conditions of heating. It having been found that *B. tetani* in pure culture develops better after heating to 70° C. for one hour than when not heated, tests were made to determine whether this also held true in the case of the organism in the presence of vaccine virus. The suspension of *B. tetani* B in dilution of 1/25,000,000 and 1/50,000,000 and a pooled specimen of 6 vaccines (L–Q) ground about 4 months previously, were used.

Twelve tubes were planted with a mixture of 1/25,000,000 dilution of the spore suspension and 0.5 c. c. of the pooled vaccine, 12 with a mixture of 1/50,000,000 dilution of spore suspension and 0.5 c. c. of vaccine, 12 controls with each dilution of the spores and 12 controls of vaccine alone. Half of the tubes were heated to 70° C. for one hour after seeding, while the remainder were left unheated.

	1/25,000,000 spore suspension+ vaccine.						1/25,000,000 spore suspension.						Vaccine 0.5 c. c. in each tube.					
Unheated.......	g^2	g^5_T	a	g^4_T	a	g^3	g^2	0	g^2	g^2	0	0	a^2	a^2	g^2_N	g^2_N	a^2	a^2
Heated to 70° C..	g^2	0	g^2	g^4_T	g^2_N	g^3	g^2	g^2	g^2	....	g^2	0	0	0	0	0	0	0
	1/50,000,000 spore suspension+ vaccine.						1/50,000,000 spore suspension.						Vaccine 0.5 c. c. in each tube.					
Unheated.......	a_T	a_N	a_T	a_N	a_N	g^2_N	0	g^2	g^2	0	0	0	a^2	a^2	a^2	g^2_N	g^2_N	0
Heated to 70° C..	0	0	g^2_N	0	0	0	g^2	g^2	g^2	g^2	g^2	0	0	0	0	0	0	0

g=gas.
a=growth in closed arm, but no gas.
0=no growth in closed arm.
Number refers to day on which gas or closed arm growth appeared.
T=positive tetanus symptoms in mice.
N=negative when tested on mice.

The results obtained in this test show that when unheated, as good or better results were obtained with a mixture of *B. tetani* spores and vaccine as with the spores alone. In heated tubes the presence of vaccine appeared to have an inhibitory effect. The control in the test with 1/50,000,000 dilution of spore suspension showed the development of spores of *B. tetani* in five out of six tubes; the mixture in none.

A further test was made with two other recently made vaccine viruses, R and S, ground two months previously, which contained a considerable number of vegetative forms of various organisms, one showing a colony count of 2,160 per c. c. and the other 2,000 per

c. c. Tests were made with a 1/50,000,000 dilution of the suspension of *B. tetani*, B, mixed with the two vaccines and planted in fermentation tubes, one-half of which were heated and half left unheated. One cubic centimeter of vaccine was planted in each tube.

	B. tetani+vaccine R.			*B. tetani* B.			Vaccine R.		
Unheated	g^{3}_{T}	g^{3}_{T}	g^{2}_{T}	0	0	0	a^{2}_{N}	a^{2}_{N}	a^{2}_{N}
Heated to 70° C	g^{3}_{T}	g^{3}_{T}	0	g^{2}	g^{2}	g^{2}	0	0	0
	B. tetani+vaccine S.			*B. tetani* B.			Vaccine S.		
Unheated	g^{4}_{T}	g^{2}_{T}	$g^{2}N$	0	0	0	a^{2}_{N}	a^{2}_{N}	a^{2}_{N}
Heated to 70° C	0	0	g^{3}_{T}	g^{2}	g^{2}	g^{2}	0	0	0

In the tubes which had not been heated, growth of *B. tetani* was not inhibited by the presence of small amounts of vaccine. On the other hand, more favorable results were obtained than with *B. tetani* spores alone, unheated, none of these tubes showing growth, while five out of six of the cultures in tubes seeded with the mixture of spores and virus produced tetanus symptoms in mice.

In the heated tubes, the presence of vaccine virus produced an inhibitory effect, so far as can be judged by the number of tubes planted. All six tubes inoculated with *B. tetani* spores alone were positive and those planted with the mixture of spores and vaccine showed three positive out of six.

Tests were carried out also with *B. tetani* C, recently isolated, together with a vaccine (K) ground five months previously, kept at incubator temperature for several months, which still contained *B. aerogenes capsulatus*, but practically no other organisms. One cubic centimeter of the vaccine was planted in each tube. A parallel test was also made with a pool of two recent vaccines, R and S. Three dilutions of the *B. tetani* spores (1/100,000, 1/500,000, and 1/1,000,000) were used and parallel tests with heated (70° C., 1 hour) and unheated tubes were run, the tubes being heated after the introduction of the vaccine, or the vaccine together with the suspension of *B. tetani* spores.

Dilution of spore suspension.	Unheated.						Heated to 70° C. 1 hour.					
B. TETANI C+VACCINE K.												
1/100,000	g^2	g^4	g^2	g^2	g^2_T	g^2_T	g^4 a^3	0	g^3	g^3	g^3	g^3
1/500,000	g^2_T	g^2_T	g^2_T	g^3_T	g^2_T	g^3_T	g^4	0	0	g^3	g^3	g^3
1/1,000,000	g^2_T	g^2_T	g^2_T	g^2_T	g^2_T	g^2_T	0	0	0	0	0	0
	18 positive, 0 negative.						9 positive, 9 negative.					
B. TETANI C+VACCINE R-S.												
1/100,000	g^3	g^3	g^3	g^3	g^3	g^3	g^2	g^2	g^3	g^2	g^{2T} a^2	g^3_T
1/500,000	g^3	g^3	g^3	g^3_T	g^3_T	g^3_T	g^3	g^3	g^2	g^2	g^3_T	g^2_T
1/1,000,000	g^8	g^4	g^3	g^4_T	g^3_T	g^3_T	0	0	0	0	0	0
	18 positive, 0 negative.						12 positive, 6 negative.					
B. TETANI C ALONE.												
1/100,000	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2
1/500,000	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2	g^2
1/1,000,000	g^2	g^2	g^2	0	g^2	0	g^2	g^2	g^2	0	0	g^2
	16 positive, 2 negative.						16 positive, 2 negative.					
VACCINE K.												
	g^{11}_N	g^6_N	g^3_N	g^4_N	g^5_N	g^3_N	a^3	a^3	g^3_N	a^3	a^3	a^5
VACCINE R-S.												
	0	g^2_N	g^3_N	g^4_N	0	0	0	0	0	0	0	

The appearance of growth and gas in the control tubes of the *B. tetani* spores alone indicated 16 positive out of 18 in both the heated and unheated tubes.

The unheated tubes, inoculated with the mixtures of the same dilution of *B. tetani* spore suspension and the two different sets of vaccine, probably were positive for *B. tetani* throughout. Not all were tested in mice, but among the 20 tested there were no negatives, while the appearance of the growth and the presence of tetanus-like organisms in smears from some of the other tubes indicated positive results. In the unheated tubes of *B. tetani* spores and vaccine virus, in 1 c. c. amounts, both in the case of an old vaccine with very few organisms, and of a freshly made vaccine with a large number of organisms, the growth of *B. tetani* and development of toxin was apparently not inhibited; indeed, the results were better than in the tubes seeded with the *B. tetani* alone.

Heating of the tubes to 70° C. after planting, in this test, however, was not as favorable to the growth of spores of *B. tetani* in vaccine virus mixtures as in pure culture, since there were 16 positives and 2 negatives among the tubes inoculated with pure cultures, 9 possible positives and 9 negatives (no growth) in the mixture with vaccine virus K, and 12 possible positives and 6 negatives (no growth) in the mixture with vaccine viruses R and S.

The following summarizes the comparative results obtained with mixtures of vaccine virus and tetanus spores heated to 70° C. for one hour, and unheated, in the above and other experiments which are not presented in detail:

B. TETANI B.

	Positive.	Negative.
Dilution 1/50,000,000+pooled vaccine viruses L–Q:		
Unheated	2	4
Heated	0	6
Dilution 1/50,000,000+vaccine virus R:		
Unheated	3	0
Heated	2	1
Dilution 1/50,000,000+vaccine virus S:		
Unheated	2	1
Heated	1	2
Total:		
Unheated	7	5
Heated	3	9

B. TETANI C.

Dilution 1/100,000+vaccine virus K:		
Unheated	6	0
Heated	5	1
Dilution 1/500,000+vaccine virus K:		
Unheated	6	0
Heated	4	2
Dilution 1/1,000,000+vaccine virus K:		
Unheated	6	0
Heated	0	6
Dilution 1/100,000+vaccine virus R–S:		
Unheated	6	0
Heated	6	0
Dilution 1/500,000+vaccine virus R–S:		
Unheated	6	0
Heated	6	0
Dilution 1/1,000,000+vaccine virus R–S:		
Unheated	6	0
Heated	0	6
Total:		
Unheated	36	0
Heated	21	15

B. TETANI C (1/1,000,000 DILUTION).

B. tetani + vaccine virus T–U:		
Unheated	6	0
Heated	6	0
B. tetani+vaccine virus V:		
Unheated	6	0
Heated	5	1
B. tetani+vaccine virus N:		
Unheated	6	0
Heated	3	3
B. tetani+vaccine virus R:		
Unheated	6	0
Heated	6	0
B. tetani+vaccine virus S:		
Unheated	6	0
Heated	5	1
Total:		
Unheated	30	0
Heated	25	5

SAME MIXTURE AS ABOVE PLANTED AFTER BEING KEPT AT 15° C. FOR 10 DAYS.

	Positive,	Negative.
B. tetani+vaccine virus T–U:		
Unheated	6	0
Heated	6	0
B. tetani+vaccine virus V:		
Unheated	5	1
Heated	4	2
B. tetani+vaccine virus N:		
Unheated	6	0
Heated	6	0
B. tetani+vaccine virus R:		
Unheated	6	0
Heated	5	1
B. tetani+vaccine virus S:		
Unheated	6	0
Heated	6	0
Total:		
Unheated	29	1
Heated	27	3

SUMMARY

Unheated	102	6
Per cent	94	6
Heated	76	32
Per cent	70	30

The results of the above tests indicate that mixtures of at least certain vaccine viruses with *B. tetani* spores produce slight, or no, inhibitory effect on toxin production when grown in plain broth and without heating after seeding, the tests being made with 0.5 to 1 c. c. of vaccine and high dilutions of spores. Results were better in general with the mixtures of vaccine and *B. tetani* spores than with *B. tetani* alone. The method of testing in plain broth fermentation tubes without heating, therefore, is apparently effective for the detection of *B. tetani* in high dilutions in vaccine virus.

Heating of tubes to 70° C. for one hour after planting with glycerinated vaccine virus and *B. tetani* spores, on the other hand, produced a number of negative results, especially in the higher dilutions of the suspension of spores, though no inhibitory effect was produced in the heating of the pure cultures.

These results emphasize the fact that it does not follow that conditions which are favorable for growth of *B. tetani* in pure culture are also favorable for the development of *B. tetani* spores when present in vaccine virus.

In most cases tubes planted with vaccine virus alone showed no growth in the closed arm after heating to 70° C. for one hour, while the corresponding unheated tubes usually showed heavy growth.

EFFECT OF VARYING AMOUNTS OF VACCINE VIRUS ON THE DEVELOPMENT OF B. TETANI SPORES.

Tests were made to determine what was the maximum amount of vaccine contaminated with a high dilution of *B. tetani* spore suspension, which could be grown in 25 c. c. fermentation tubes without inhibiting the growth of the tetanus organism. Tubes were inoculated with mixtures of the same amount of a suspension of spores and varying amounts of vaccine virus. Suspension C in a dilution of 1/500,000 was used and vaccine virus R in amounts of 0.5 c. c., 2 c. c., 5 c. c., and 10 c. c. per tube. These were run in parallel sets of heated and unheated tubes.

B. TETANI C (1/500,000) AND VACCINE VIRUS R.

	Unheated.			Heated to 70° C. 1 hour.		
B. tetani+1/2 c. c. vaccine	g^{5}	g^{3}	g^{2}	g^{2}	g^{2}	g^{3}
B. tetani+1 c. c. vaccine	g^{11}_{T}	g^{4}_{T}	g^{4}_{T}	g^{4}_{T}	0	g^{3}_{T}
B. tetani+2 c. c. vaccine	g^{5}_{T}	g^{4}_{T}	g^{4}_{T}	g^{3}_{T}	0	0
B. tetani+5 c. c. vaccine	a^{4}_{N}	a^{4}_{N}	a^{4}_{N}	0	0	0
B. tetani+10 c. c. vaccine	a^{3}_{N}	a^{6}_{N}	a^{6}_{N}	0	0	0
B. tetani (1/500,000), no vaccine	g^{2}	g^{2}	0	g^{2}	g^{2}	g^{2}

Mice were inoculated with cultures from tubes showing growth in the closed arm, with or without gas, except those planted with ½ c. c. of vaccine. Positive results were obtained in mice inoculated from tubes which contained 2 c. c., or less, of the vaccine. Tubes containing 5 c. c. and 10 c. c. of vaccine showed growth in the closed arm of the unheated tubes, but no gas, and the animal tests were negative. The heated tubes inoculated with these amounts showed no growth and no gas in the closed arm of any. An amount of this vaccine, therefore, not exceeding 2 c. c. planted in 25 c. c. fermentation tubes seems to be favorable for the detection of *B. tetani.*

Attempts were also made to detect small amounts of tetanus spores in large amounts of vaccine virus, by planting 20 c. c. of contaminated virus in Kjeldahl flasks containing 500 c. c. of plain broth. The results, however, were so irregular that this method was abandoned.

EFFECT OF OTHER SPORE-BEARING ORGANISMS ON THE GROWTH OF B. TETANI.

A number of cultures were isolated from flasks planted with the cut-off points mentioned in the preceding section, with the hope of isolating the *B. tetani* like organisms which frequently appeared in smears. The method used was to plant high dilutions of the broth cultures in melted deep agar tubes, allow these to harden, and after incubation break off the tube at the points where favorable colonies appeared. Transfers from such colonies were made to broth fermentation tubes.

On growth in broth after isolation, none of the organisms isolated resembled *B. tetani* closely enough morphologically to be confused therewith by an experienced observer.

The effect of a number of these organisms grown in association with spores of *B. tetani* was studied, but none showed any unusual properties except two designated as E4 and A3—E4 gram-positive rods, with slightly elongated terminal spores; A3 gram-positive rods, with terminal oval spores.

The culture E4 produced about the same amount of gas in plain broth as *B. tetani*, but showed a slightly heavier growth in the closed arm of the fermentation tubes. It appeared to be anerobic by preference, but grew also in the open arm of the Smith tube after one or two days. Six tubes were planted with this culture and 1/50,000,000 dilution of *B. tetani* B. The culture from only one produced tetanus symptoms in a mouse inoculated with 1/10 c. c., five other mice inoculated with 1 c. c. failed to develop symptoms. The control tubes of *B. tetani* alone showed five positive out of six. The organism apparently had an inhibitory effect on the production of tetanus toxin.

Further tests were carried out with the same organism grown in association with the same and other *B. tetani* cultures, using 0.1 c. c. of culture E4 in each tube.

	B. tetani A (1/100,000)+E4.						B. tetani A (1/100,000).					
Unheated	g^1_T	g^1_T	g^1_T	g^1_T	g^1_T	g^1_N	0	g^3	g^2	g^3	g^5	g^5
Heated to 70° C. (1 hour)	g^1_N	g^1_N	g^1_T	g^1_T	g^1_N	g^1_T	g^2	g^2	g^2	g^2	g^2	g^2

	B. tetani B (1/25,000,000)+E4.						B. tetani B (1/25,000,000).					
Unheated	g^1_N	g^1_N	g^1_N	g^1_N	g^1_N	g^1_N	g^2	g^2	0	g^2	g^2	g^2
Heated to 70° C. (1 hour)	g^1_T	g^1_T	g^1_N	g^1_T	g^1_N	g^1_N	g^2	g^2	g^2	g^2	g^2	g^2

	B. tetani C (1/500,000)+E4.						B. tetani C (1/500,000).					
Unheated	g^1_T	g^1_N	g^1_N	g^1_N	g^1_T	g^1_N	0	g^2	0	g^2	g^2	g^2
Heated to 70° C. (1 hour)	g^1_T	g^1_T	g^1_N	g^1_N	g^1_N	g^1_N	g^2	g^2	g^2	g^2	g^2	g^2

Tests on animals were made at the end of 6, 9, and 15 days. Of 12 tested at the end of 6 days, 3 were positive, 9 negative. Of 12 other cultures tested at the end of 9 days, 5 were positive and 7 negative, and of the remaining 12 tested at the end of 15 days, 7 were positive and 5 negative. Seven of the tubes, negative at the end of 6 and 9 days, were tested again at the end of 15 days and again found negative.

Of the 36 control tubes inoculated with pure cultures of *B. tetani* 32 showed the typical appearance for tetanus growth and four showed no growth. Two of the positive tubes produced symptoms in mice, and since throughout the work it has always been found that tubes inoculated with pure cultures which show growth and typical appearance always produce tetanus symptoms in animals, it may be assumed that the 32 tubes showing growth were all positive and the four showing no growth were negative. Of the 36 tubes inoculated with mixtures of the organism E4 and the same amount of *B. tetani* suspensions as were used in the control tubes, 15 caused tetanus in mice, 21 were negative with 1 c. c. doses.

Cultures of the organism E4 itself had no effect on mice in 1 c. c. amounts. The results obtained show that it overgrew *B. tetani* or inhibited toxin production in about 60 per cent of cases.

The culture A3 produced no gas in plain broth, and grew in the closed arm of the fermentation tube, but produced a considerably heavier growth than *B. tetani*. Growth also later extended into the open arm. Grown in association with *B. tetani* B, 1/50,000,000 dilution, it inhibited gas production. Three tubes showing no gas produced tetanus symptoms in mice. Further tests were carried out with other tetanus cultures, final readings being made on the seventh day.

	B. tetani C (1/500,000)+1/10 cc. A3.						B tetani C (dilution 1/500,000). (All showed gas, which about filled the dome of the tube.)					
Unheated	a^1	a^1	a^1	a^1	a^1_T	a_T	g^3	g^2	g^2	g^3	g^2	g^2
Heated to 70° C	a^1	a_N	g^1[a]	g^1[a]	g^1[b]	a^1_T	g^2	g^2	g^2	g^2	g^2	g^2

[a] Very small bubbles. [b] Bubble not quite filling dome of tube.

Mice were inoculated with 1/10 c. c. from four different tubes, showing no gas and tetanus symptoms were produced in three. A mouse was also inoculated with 1 c. c. from the fourth tube, but failed to develop tetanus.

	Tetanus A+1/10 c. c. A3 1/100,000.						Tetanus A (producing a larger amount of gas than other tetanus cultures), 1/100,000.					
Unheated	a^1	a^1	a^1	a^1	a^1_T	a^1_T	g^2	g^2	g^2	g^2	g^2	g^2
Heated to 70° C	g^1	g^1_T	g^1	g^1_T	g^1	g^1	g^2	g^2	g^2	g^2	g^2	g^2
	No gas in any of the unheated tubes. Small bubbles, some scarcely visible, and 1 which nearly filled dome of tube, present in the heated tubes.						All showed ½ inch of gas, the unheated tubes perhaps slightly less, and the heated slightly more.					

Tetanus symptoms were produced in mice inoculated with 1/10 c. c. of cultures from four tubes, two of which showed no gas, and others very small bubbles.

Several instances occur throughout the work in which positive symptoms were produced in animals inoculated from tubes planted with mixtures of tetanus spores and vaccine virus in which growth in the closed arm without gas was present. It is possible that such results may have been due to organisms similar to that just described.

It seems advisable, therefore, in testing for *B. tetani* in vaccine virus, that cultures from all tubes which show growth in the closed arm be injected into animals.

TIME OF APPEARANCE OF GAS IN CULTURES.

The time of the appearance of gas in fermentation tube cultures of *B. tetani* in plain broth is so variable that we have compiled here the data from all of our experiments. The compilation includes the cultures of *B. tetani* alone and cultures of the organism mixed with vaccine virus.

Day of appearance of gas.	Number of fermentation tubes planted with *B. tetani* showing gas.	Number of fermentation tubes planted with *B. tetani* and vaccine showing gas.
1 day	17	2
2 days	311	76
3 days	93	126
4 days	31	39
5 days	25	[illegible]
6 days	8	3
7 days	10	4
8 days	1	0
9 days	1	0
10 days	0	1
11 days	1	2
12 days	1	1
13 days	1	0
14 days	0	0
15 days	0	0
16 days	1	0

Gas production in tubes planted with vaccine and *B. tetani*, it should be understood, does not necessarily indicate the development of *B. tetani*. The highest percentage of positive results in the case of *B. tetani* alone were obtained in 48 hours, though gas production as late as seven days after planting was not infrequent, and once a period of 16 days elapsed before the typical appearance for *B. tetani* was evident. In the case of mixtures of vaccine and *B. tetani* the highest percentage of positives was shown three days after planting.

EFFECT OF THE PRESENCE OF B. AEROGENES CAPSULATUS ON THE DEVELOPMENT OF B. TETANI SPORES.

Negative results for *B. tetani* were obtained in tests made by planting in Kjeldahl flasks 20 c. c. of vaccine virus known to be contaminated with *B. aerogenes capsulatus* and artificially contaminated with *B. tetani* spores. For this test a vaccine was used which had been kept at warm-room temperature, and which contained *B. aerogenes capsulatus* and but few vegetative forms. Control flasks planted with *B. tetani* spores alone (culture B, 1/25,000,000) were positive, while the flasks planted with the vaccine were negative and were found to contain *B. aerogenes capsulatus.*

Judging from these results obtained in flasks, and the negative results obtained in mice inoculated with several cultures from fermentation tubes showing a large amount of gas, it was thought possible that the presence of *B. aerogenes capsulatus* at times interfered with the development of *B. tetani.*

Tests were therefore made with suspension B in a dilution of 1/50,000,000, which was planted alone in 12 fermentation tubes as controls, and also in 12 tubes which were each later planted with a loopful of broth culture of *B. aerogenes capsulatus.* Half of the tubes were unheated and half were heated to 70° C. for one hour.

B. TETANI SPORES AND B. AEROGENES CAPSULATUS.

Unheated	g^{1}_{T}	g^{1}_{T}	g^{1}_{T}	g^{1}	g^{1}	g^{2}
Heated 70° (1 hour)	g^{1}_{T}	g^{1}_{T}	g^{1}_{T}	g^{1}	g^{1}	g^{1}
	(About 1 inch of gas in all)					

B. TETANI SPORES ALONE

Unheated	0	0	0	0	0	0
Heated 70° (1 hour)	g^{2}	g^{2}	g^{2}	0	0	g^{7}

In this test it was evident that growth of *B. tetani* and production of tetanus toxin in fermentation tubes were not inhibited by the presence of *B. aerogenes capsulatus*, but, on the other hand, it was favored in unheated tubes planted with the two organisms.

It appears, therefore, from this test, made in fermentation tubes, that the presence of *B. aerogenes capsulatus* artificially introduced did not necessarily inhibit the growth of *B. tetani* and that the failure of *B. tetani* to develop in the flasks was due to other factors. This agrees with the observations of Francis and makes it plain that some other explanation must be sought for the negative results obtained with tubes showing much gas.

ISOLATION OF B. TETANI IN PURE CULTURE.

The following method was used in the isolation of *B. tetani* in pure culture from several of the mixed cultures mentioned in the preceding section (pp. 5–12).

Dilutions of 1/100 to 1/1,000,000 were made from positive cultures from 4 flasks which had been planted with "ivory" vaccine points and from a fermentation tube culture which had produced tetanus symptoms in animals. These dilutions were planted in melted, deep agar tubes and heated for one hour to 70° C. The tubes were then allowed to solidify and were incubated. After three days' incubation colonies that appeared favorable were isolated by breaking off the tube at the point where these appeared and fishing to plain broth fermentation tubes. The breaking of the culture tube was accomplished by making a scratch all around the tube with a file and pressing a glass rod heated to redness against the scratch mark.

TESTS ON ANIMALS BY DIRECT INOCCULATION OF VACCINE VIRUS ARTIFICIALLY CONTAMINATED WITH B. TETANI SPORES.

Tests were made by injecting guinea pigs directly with *B. tetani* spores (*B. tetani* C in dilution 1/100,000, 1/500,000, 1/1,000,000) and the same dilutions mixed with vaccine virus K, relatively free from other organisms. Those receiving tetanus alone received 1 c. c. of the suspension and others received 2 c. c. of the mixture, subcutaneously, i. e., the same quantity of suspension of spores. None developed tetanus symptoms within 10 days. Corresponding plain broth fermentation tubes, inoculated with the same amounts of suspension plus vaccine practically all developed tetanus toxin, as shown by tests of the cultures on mice.

Further tests were made with *B. tetani* C in a dilution of 1/1,000,000 mixed with several different recently made vaccines (N, R, S, V, W, X) which contained staphylococci and other organisms (colony counts 460, 2060, 930, 570, 460, and 400 per cubic centimeter, respectively). Mixtures were made so that 0.5 c. c. contained 1/1,000,000 of the suspension of spores. Guinea pigs were injected subcutaneously with 0.5 c. c. of the mixture. None developed symptoms during 14 days' observation. Plain broth fermentation tubes seeded with corresponding suspensions of spores plus vaccine developed tetanus toxin. A series of six tubes was planted with ½ c. c. of each mixture practically all showed typical *B. tetani* growth, and one of each series tested on mice produced tetanus symptoms.

Two sets of the contaminated vaccine viruses referred to in the preceding paragraph were stored at cold-room temperature (15° C.) and warm-room temperature (37° C.) and tests repeated on animals at the end of certain periods to determine the possibility of the development of tetanus toxin in vaccine. Tests were made on guinea pigs with 0.5 c. c. of the contaminated viruses after 7 days' incubation at warm-room temperature. None developed tetanus symptoms during 14 days' observation, though, as before, mice injected with broth cultures of the same contaminated vaccines developed symptoms of tetanus. A third test was made on mice by direct inoculation after 12 days' further incubation of the vaccines, i. e., after 19 days' storage, but tetanus symptoms were not produced in any of the animals.

Further tests were made by injecting guinea pigs with mixtures of a recent vaccine (1 month old) and lower dilutions of *B. tetani* spores C, as well as with the dilution used above 1/1,000,000. One cubic centimeter of the mixture was injected in each animal. Tetanus symptoms developed in the guinea pigs inoculated with the virus contaminated with 1/1,000 c. c. and 1/10,000 c. c. of the suspension of spores, but not in those injected with 1/100,000 and 1/1,000,000. Two pigs were injected with each dilution and similar results were obtained with both in each set.

All control fermentation tubes seeded with the same amounts of mixture showed the development of tetanus toxin as demonstrated by the animal test in all dilutions used.

The results obtained showed that it is possible to detect a very much smaller amount of *B. tetani* contamination, both alone and in vaccine virus, by cultural methods and animal inoculation from the culture than by direct animal tests.

THE STANDARDIZATION OF PITUITARY EXTRACTS.

By Reynold A. Spaeth, Technical Assistant, Hygienic Laboratory.

In the following paper the present status of the problem of the biological assaying of pituitary extracts will be discussed and certain theoretical and practical objections to the method which is recognized by the U. S. P. IX will be pointed out. Evidence of the advantages of using a simplified standard, potassium chloride, which has been investigated in both its theoretical and practical aspects will also be presented. Finally the results of a comparative study upon seven commercial preparations of pituitary extracts will be reported.

HISTORY OF THE PRESENT METHOD.

Dale and Laidlaw (1912) first suggested using the isolated uterus of the virgin guinea pig as a test organ for determining the strength of pituitary extracts, though Kehrer (1907) had previously used this organ for estimating the physiological activity of ergot preparations. Heidelberg, Pittenger, and Vanderkleed (1914) verified the claims of Dale and Laidlaw and were thoroughly convinced that the uterine method was superior to the blood pressure technique. Subsequently Roth (1914*a*) carried out a detailed investigation of the isolated uterus method and reported the great variability in the physiological activity of 11 commercial samples of pituitary extract purchased in the open market. Roth appears to have been the first investigator thoroughly to grasp the theoretical and practical importance of finding a suitable standard substance having a qualitative effect upon the isolated uterus comparable to that of pituitary extract. Until the appearance of Roth's publication all the methods of "standardization" had been essentially comparative. This fact would seem to account for the high degree of variability in the commercial products which Roth described, though it should be emphasized that a comparison of two or more samples from the same manufacturer showed a satisfactory constancy of physiological action. In the hope of increasing the uniformity of commercial pituitary extracts, Roth (1914*b*) suggested the use of β-iminazolylethylamine hydrochloride (histamine) as a standard substance with the physiological effects of which the reactions to pituitary extracts of unknown strength were to be compared. This suggestion was subsequently incorporated into the U. S. P. IX.[1]

OBJECTIONS IN THE LITERATURE TO THE U. S. P. RECOMMENDATION.

Numerous objections, chiefly by representatives of the research laboratories of various manufacturing concerns, have been advanced against Roth's method and standard. These objections fall into three groups. (1) The character of the test reaction has been questioned. Hamilton (1917) states: "While the method is apparently simple, the test strips of uterus muscle which will respond differently to different sized doses with any degree of accuracy are so few that a dependable test can rarely be made." According to Hamilton and Rowe (1916): "The isolated uterus test, while in some respects logical, is not in its present form practicable to be used as a commercial method for the final standardization of pituitary extract." Somewhat more conservative criticisms of the uterus method have been advanced by Fenger (1916), who states: "The selection of a suitable uterus is not always an easy matter. Often three or four guinea pigs have to be killed before a satisfactory strip is obtained." It appears that the sweeping condemnation of the uterus method as expressed by Hamilton and Rowe finds little support among the manufacturers, since at least five commercial laboratories are now using this method for testing pituitary extracts. Our own experience confirms that of Fenger, i. e., both hyper- and hyposensitive uterine strips frequently appear in the course of the experiments. These anomalous conditions necessitate a carefully conducted series of tests on successive days if concordant and valid results are to be obtained. Thus, whereas the technical manipulation involved in the uterus method is exceedingly simple, the correct interpretation of the results requires experience and invariably involves a personal equation of considerable magnitude.[2]

However, in spite of the varying responses which occur in different preparations, the high sensitivity of the uterus to pituitary extracts and the fact that pituitary extract finds one of its chief applications in cases of uterine inertia, make the retention of the method appear advisable.

(2) Objections have also been advanced against the use of histamine as a standard substance with which to compare the physiological activity of pituitary extracts. Thus Pittenger and Vanderkleed (1917) state that: "Before adopting a complex substance like the

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[1] On page 247 of the U. S. P. IX under "Liquor hypophysis" we read: "One mil of solution of hypophysis, diluted 20,000 times, has the same activity on the isolated uterus of the virgin guinea pig as a 1 to 20,000,000 solution of beta-iminazolyl-ethylamine hydrochloride when tested as directed by the United States Hygienic Laboratory."

[2] We have recently suggested a more objective method for pituitary standardization. Spaeth, R. A., J. Pharm. and Exp. Ther. XI. 209.

above (histamine) as a standard for adjusting the strength of commercial preparations, it would have been better, perhaps, to make a thorough study of a number of problems such as the following:

"1. Degree of uniformity in the physiologic action of different available samples of the proposed standard substance.

"2. Rate of deterioration of solutions of this substance.

"3. Effect of sterilization on solutions of this substance.

"4. Rate of deterioration of the substance itself.

"5. Effect of repeated doses on the isolated uterus.

"6. The toxicity of the substance as compared with pituitary extract.

"7. The relative activity of a pituitary extract of the strength proposed by the U. S. P. IX and that of the commercial extracts as supplied by the leading pharmaceutical manufacturing houses."

In justice to Roth and the revision committee of the U. S. P. IX, it should be pointed out that Roth investigated the above problems with the exception of Nos. 4 and 6. He found (*a*) different available samples of histamine had equal physiological effects when compared upon the same uterine strips (fig. 4, p. 569, J. Pharm. and Exp. Therap. 1913–14, V); (*b*) no deterioration occurred in standard solutions of histamine of 1:1000 for about six months when kept in a cool dark place; (*c*) sterilization on three successive days at 100° C. for 20-minute periods had no appreciable effect on the standard solution when its activity was determined on the isolated uterus of the virgin guinea pig (Roth, l. c. p. 25). Roth also investigated the effect of clear and amber glass ampoules upon histamine.

Appreciating the practical difficulties of making up a fresh 1:20,000,000 solution of histamine for each experiment, Roth (1914) states (p. 478): "A 1:1,000 solution of it was made, put into sterile ampules which held a little over 1 c. c. of solution and subsequently sterilized fractionally. A fresh 1:20,000,000 solution made from the sterilized 1:1,000 solution was used as a standard in each experiment." In spite of the fact that Pittenger and Vanderkleed (l. c.) call attention to the publication from which this quotation is taken, they state (p. 31): "We thought it advisable to determine whether or not it would be possible to prepare a 1:1,000 dilution of the salt, put this up in ampules and then dilute the contents of one of these ampules to 1:20,000,000 for each test."

Although Pittenger and Vanderkleed's paper indicates a most superficial perusal of the literature, they have brought out one very significant fact, namely (p. 132), that "new solutions of the original substance possessed only about one-third of the activity of the solutions prepared one year before from the same sample," which led to the conclusion that the dry salt lost its physiologic activity at a greater rate than solutions of the salt preserved in ampules. This observation agrees with the experience of the writer who has found that samples of histamine which have been on hand for about three years had, apparently as the result of some sort of spontaneous molecular change, lost a considerable part of their physiological activity. Such a spontaneous degeneration on the part of histamine constitutes a most serious objection to its use as a "standard" for assaying pituitary extracts; and this fact may account for some of the difficulties encountered in its use in assaying pituitary extracts. This point will be reverted to further on.

(3) Finally it is claimed that the standard adopted by the U. S. P. IX is too low. Hamilton (l. c.) estimates that it is 60 per cent too low, Pittenger and Vanderkleed (l. c.) 300 to 500 per cent too low, and Eckler (1917) 1,000 per cent too low. Eckler states: "The results of my work in the subject are so utterly at variance with the pharmacopœial statement that I am prompted to question whether or not the requirement as stated is correct." The pharmacopœial requirement states very definitely that the uterine test is to be carried out "as directed by the United States Hygienic Laboratory." Roth (1914a) describes his technique in great detail and states (p. 23) that "young pigs weighing from 275 to 350 grams should preferably be used." Again, Roth states (p. 20) that his tests were carried out on "a piece of uterus measuring from 1 to 2 centimeters." Eckler used "the uteri of virgin guinea pigs weighing from 175 to 250 grams," finding that "larger pigs up to 300 grams" proved less satisfactory. Furthermore, Eckler worked with the entire horn, unless it was unusually long, in which case a part of the ovarian end was removed.

Eckler's independence of the pharmacopœial regulations is not unique. Indeed, we find different investigators advocating the use of virgin guinea pigs varying from 175 to 425 grams.

Similar nonconformity appears in the choice of uterine material. Uterine strips 1–2 cm. and 2.5 cm., whole uterus horns without ovaries, whole horns with ovaries, whole horns with indeterminate fractions of the ovarian end cut off, and both horns of one uterus tied together have all been used in testing pituitary extracts.

With such variations in technical manipulation, coupled with the undoubted gradual spontaneous deterioration of histamine, it is not surprising that attempts to standardize pituitary extracts vary widely in their results.

The manufacturers evidently feel that obstetricians and surgeons have little faith in the pharmacopœial standard, suspecting it to be too weak. They have consequently adopted the entirely illogical and theoretically untenable practice of labeling pituitary extracts "stronger than the U. S. P." or "at least as strong as the U. S. P.," which is both indefinite and dangerous.[1] In the case of a relatively powerful drug like pituitary extract a standard becomes perfectly futile if it fails to determine an upper limit of concentration. The main purpose of insisting upon a definite strength of drug is to produce uniform products and to protect patients against the toxic effects of overdoses. However, it is obvious that in obstetrical practice, where time is frequently an important factor, all uneffectual concentration is quite as undesirable, though possibly not so dangerous as an overdose. It is, therefore, essential to establish a limit of concentration of pituitary extract which will produce contractions approximately equal to the contractions produced by a stable standard substance. It is perfectly natural that the manufacturers should supply the medical profession with drugs of a strength which approximates that to which they are accustomed. On the other hand, it is highly desirable to eliminate the present variations in the commercial pituitary samples, and especially to control the production of extracts more than 10 times as strong as the U. S. P. IX (Eckler, 1917, p. 201).

THE SELECTION OF A STANDARD.

It is evident from the above review of the recent literature on the standardization of pituitary extracts that the gradual deterioration of beta-iminazolylethylamine hydrochloride presents a very serious objection to its use as a standard. The writer's experiments confirm the evidence that histamine, on account of its deterioration and for other reasons, is not a practical standard. It has been found that the melting point of supposedly pure samples of histamine showed striking irregularities and that recrystallizations were accomplished with considerable difficulty. The melting point determinations and recrystallizations were carried out by Dr. C. N. Myers. It was concluded that the samples had deteriorated in the course of about three years and that a single chemically pure substance was no longer being dealt with. Furthermore, certain samples which had been kept in cork-stoppered bottles showed a discoloration of the cork, suggesting a decomposition of the histamine. When tested physiologically these samples were found to be relatively weak.

In addition to the gradual deterioration of the drug there are several practical objections to the use of histamine as a standard.

(1) Owing to war conditions, the European supply of histamine is completely cut off and the substance has consequently become both difficult to obtain and expensive.

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[1] Roth and others have recorded numerous cases of ruptured uterus resulting from an overdose of pituitary extract.

(2) Since the virgin guinea-pig uterus is so extremely sensitive to histamine, responses having been observed in a dilution of 1:250,000,000, there are chances for relatively great errors in making up the requisite dilutions.

(3) The salt is hygroscopic, which involves an appreciable error in weighing out 100 mg.

(4) The standard solution must be preserved in sealed and sterilized ampules, a procedure involving considerable time and labor.

The question naturally follows: "What are the prerequisites of an ideal standard for the assaying of pituitary extracts?"

(1) An ideal standard must produce a qualitative effect upon the isolated uterus, a contraction, which is relatively constant, completely reversible, and therefore noninjurious to the preparation.

(2) The relative sensitivity of different uterine strips must rise or fall equally and not independently when tested by the standard and pituitary extracts. Thus low sensitivity in a given strip should obtain both to pituitary extract and to the standard. This factor is of fundamental importance in the selection of a standard substance.

(3) An ideal standard should be highly stable under ordinary working conditions, and easily obtainable in chemically pure form. Any complex organic substance because of the intricacy of its molecular structure affords a greater opportunity for spontaneous change at ordinary temperatures than does a substance of such simple molecular structure as sodium or potassium chloride. The selection of a simple inorganic salt as a standard would thus present distinct theoretical advantages over most organic substances.

(4) Finally, the ideal standard should be a substance to which the sensitivity of uterine preparations is not excessively high because of the increased chance of error in making the required dilutions.

THE EFFECT OF POTASSIUM CHLORIDE UPON THE ISOLATED GUINEA-PIG UTERUS.

Consideration of the foregoing theoretical advantages of a simple inorganic substance as a standard in assaying pituitary products led to an investigation of the effects of potassium chloride upon the isolated uterus.

It has recently been shown (Spaeth, 1918) that potassium chloride has a qualitative effect comparable to that of pituitary extracts upon the melanophores of the minnow *Fundulus heteroclitus*. Since it had previously been found that the melanophores are functionally modified smooth muscle cells (Spaeth, 1916) and contract in solutions of potassium chloride (Spaeth, 1913) it seemed highly probable that uterus strips would also contract when immersed in solutions of KCl. Experiment confirmed this supposition.

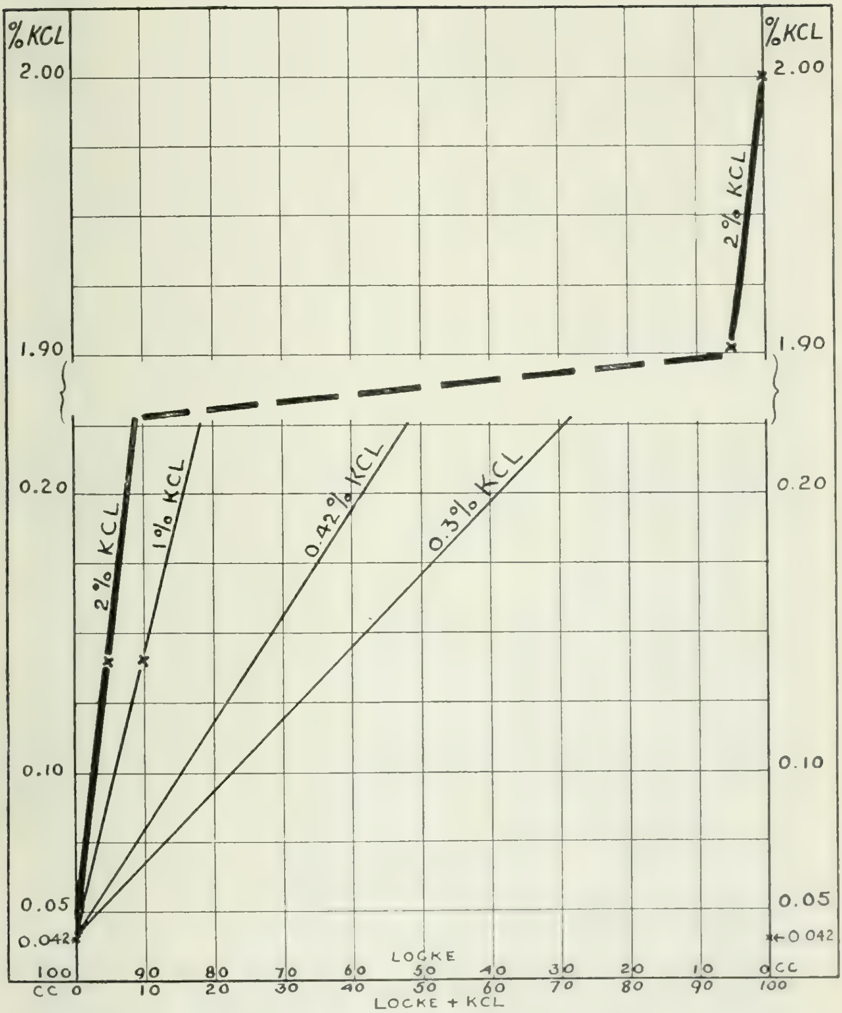

Fig. 1.—Graph showing the method of obtaining the potassium standard solution of 0.14 per cent KCl in Locke from Locke solutions of different potassium content. A 2 per cent concentration of KCl was found most practicable.

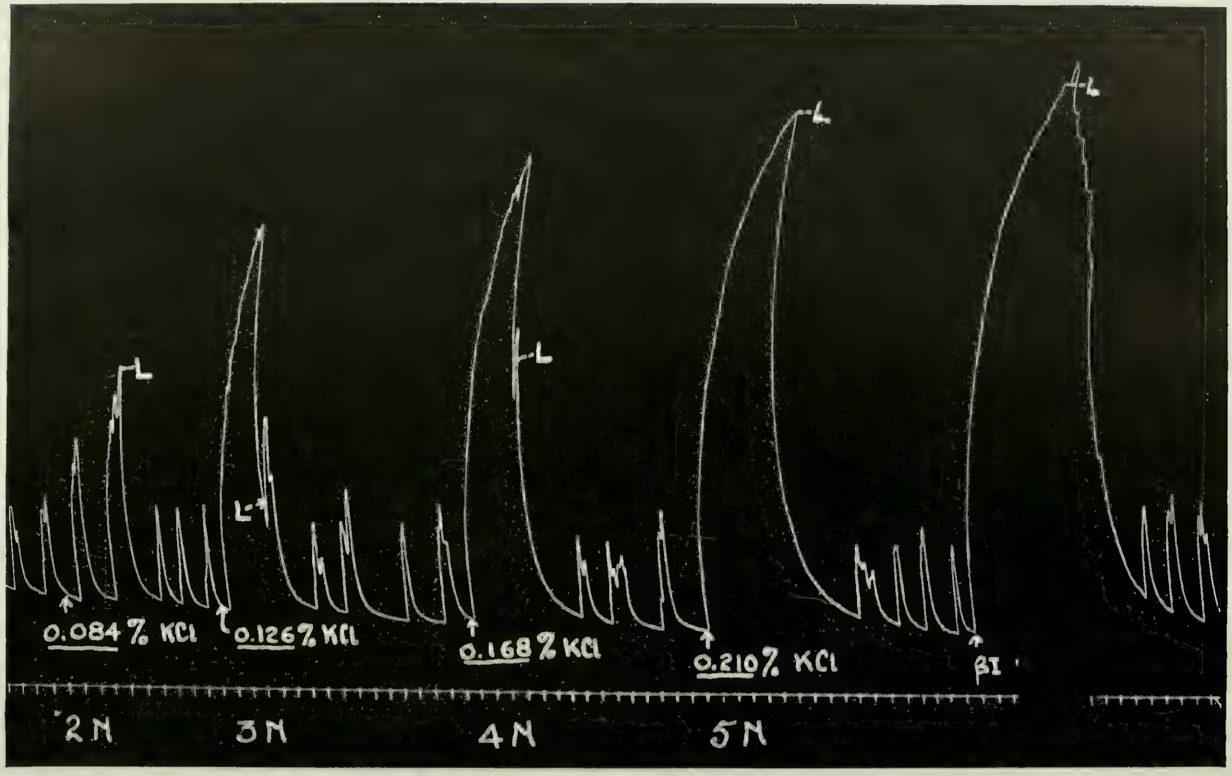

Fig. 2.—Tracing showing the effects of potassium chloride in doses two, three, four, and five times normal. Time=minutes; L=Locke.

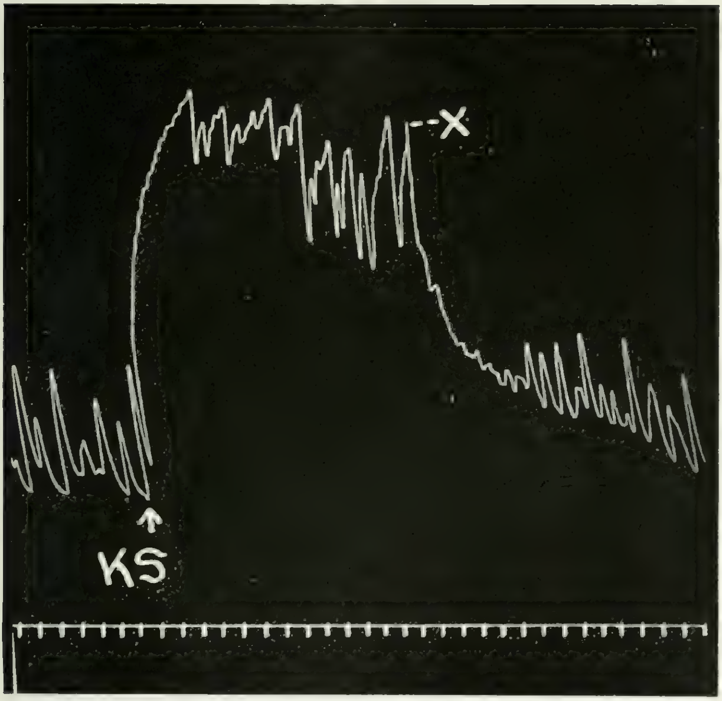

Fig. 3.—Tracing showing the effects of a prolonged action of the potassium standard solution. Time=minutes; x=Locke.

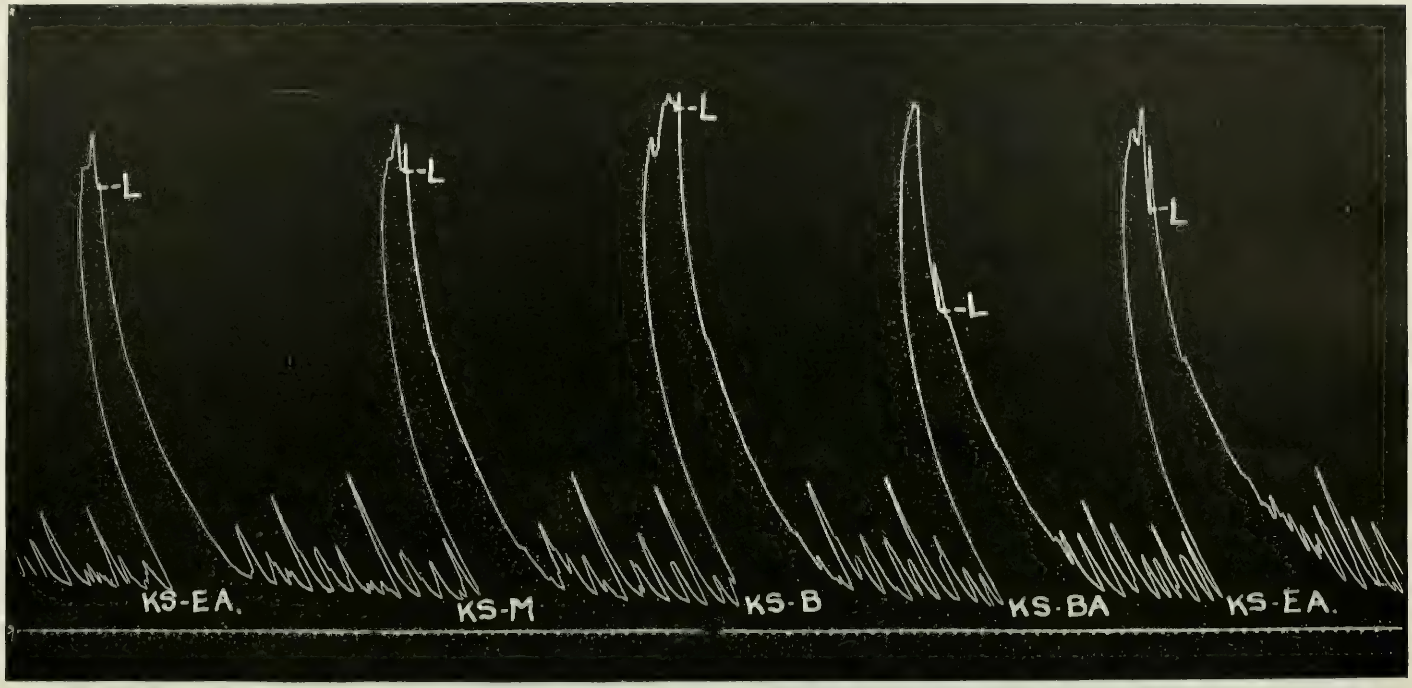

Fig. 4.—The comparative effects of four samples of KCl at a concentration of 0.14 per cent in Locke. L=Locke; time=minutes; other letters refer to names of the manufacturers.

METHOD.

The experimental procedure was similar to that of Roth. The thermostat was slightly modified in that it contained two glass receptacles in each of which a uterine strip was suspended.

Used solutions were removed by means of stopcocks below the thermostat, but fresh Locke solution was always added at the upper opening by carefully pouring in a measured amount of the liquid from a beaker, previously quickly heated to 39° C.

Small quantities of the potassium chloride and the pituitary extracts were added as cold concentrated solutions from carefully cleaned and accurate pipettes. The same pipette was used for each solution throughout the experiments. The usual precautions were carefully observed in the matter of glass-distilled water and c. p. chemicals in making up the Locke solution. We used the formula for Locke's solution given by Roth, and fresh Locke was made up daily from concentrated solutions of the constituent salts.

The total concentration of potassium chloride used in any given experiment was read off directly with the aid of a simple graph, figure 1. After a preliminary series of empirical trials, we finally adopted a Locke solution containing 2 per cent KCl, in place of the normal 0.042 per cent KCl, as a stock solution. Varying amounts of this KCl stock were added to normal Locke solution, but in every case the total volume was 100 c. c. By this procedure the KCl was the only variable introduced and the results were consequently strictly comparable. Raising the concentration of potassium chloride in a Locke solution from 0.042 to 0.14 per cent obviously increases the osmotic pressure of the solution considerably. In order to determine whether the contraction produced in the uterus by KCl might involve an osmotic factor in addition to the specific stimulating effect, the effects of adding equivalent concentrations of NaCl and KCl alternately to the same preparation were tested. The only observable effect produced by the NaCl was a slight lengthening of the interval between rhythmic contractions. The height of the contractions was never affected. Since the total osmotic pressure was approximately the same in the two abnormal solutions, the contraction appearing in the KCl solution is due to a specific salt effect and not to an increase in osmotic pressure. The above tests with NaCl, as well as additional experiments in which 5 c. c. of cold, normal Locke solution were added to the 95 c. c. in the suspension chamber, showed that 5 c. c. of cold solution failed to produce any characteristic effect and never a high contraction like that brought about by potassium chloride.

EXPERIMENTAL RESULTS.

Figure 2 shows the effects of gradually increasing doses of KCl upon a normal strip of virgin guinea-pig uterus. Doubling the amount of KCl normally occurring in Locke solution causes an increased amplitude of the spontaneous rhythm. Additional concentrations of potassium chloride produce a slow and gradual contraction, which, however, does not become maximal until a concentration of about five times normal (0.21 per cent) is attained. Upon substituting Locke's for the potassium solution, an immediate and rapid relaxation normally occurs, which is soon followed by the reappearance of spontaneous rhythmic contractions.

From the percentage of KCl recorded in the figure we can see that concentrations of 0.168 per cent KCl or less produce distinctly submaximal contractions. Further experiments verified this observation repeatedly. We finally selected a Locke solution having a total concentration of 0.14 per cent KCl as a standard with which to compare the physiological effects of pituitary extracts. We shall hereafter refer to this solution as the potassium standard. It is noted in the figures as KS.

A study of the contractions 3N and 4N in figure 2 show that after a maximal height had been attained for a given concentration there was a sharp drop toward the base line. That the appearance of this drop followed the actual high point in a contraction was proved by studying the effects of the prolonged action of the potassium standard. Figure 3 shows that after a considerable rise in tone damped rhythmic contractions appear, which gradually increase in height, with an accompanying drop in tonus. The return to Locke solution (at x) causes a sharp drop in tone, followed by a slow reappearance of the normal rhythm.

Although we might expect different samples of potassium chloride to have identical physiological effects upon a single uterus strip, nevertheless the apparent effect of the potassium solution might be due to some contaminating impurity. We therefore tested the effects of the following four samples of commercial potassium chloride:

1. Merck's "Blue Label"; one-fourth pound, No. 11685 (0.7311).
2. Baker's "C. P."; a 500-gram sample of lot No. 11013.
3. Baker and Adamson's "C. P."; 1 pound of lot No. 3772.
4. An old sample of Eimer and Amend's potassium chloride, which had evidently been on hand a long time, judging by the discoloration of the label. The degree of purity was not stated on the container.

A typical result of one of several tests made with these four salts is shown in figure 4. In every case the potassium standard (KS) concentration was used. The letters refer to the names of the manufacturers. The figure illustrates the typical gradual increase in general sensitivity, which always appears in a uterus preparation in the course of several hours. The greatest variations produced in the contractions by different salts are of the same order (ca. 6 per cent) as the difference between the first and last contractions, produced by the same sample of potassium chloride. Our tests have thus shown that any of the samples of KCl listed above may safely be used in making up a potassium standard solution. Furthermore, the age of a preparation of potassium chloride appears to have no influence on its physiological potency.[1]

A single figure will suffice to show the qualitative similarity of physiological effect produced by potassium chloride and histamine (fig. 5). In this case the histamine appears to have deteriorated somewhat. Both the potassium and the histamine solutions produced a submaximal contraction when compared with a strong solution of pituitary extract. In both cases recovery was rapid and complete.

COMPARATIVE STRENGTHS OF COMMERCIAL PITUITARY EXTRACTS.

(1) DESCRIPTION OF SAMPLES.

The practicability of using potassium chloride as a standard for comparing the physiological potency of pituitary extracts having been determined, a comparative study of seven commercial pituitary preparations was made. The preparations were all aqueous extracts of the infundibular portion of the pituitary gland. They were procured in the open market and were kept in the original packages in a relatively cool and dark place. The following samples were investigated:

Sample I. Solution "Pituitary extract Mulford"; made by the H. K. Mulford Co.; serial No. 330885A; dispensed in clear glass ampules containing about 1 cubic centimeter of a clear, colorless fluid having a strong odor of camphor. This sample was labeled "Stronger than the U. S. P. 1 mil represents 0.2 gm. of fresh posterior lobe. We do not recommend the use of this product after April 9, 1919." In a leaflet inclosed in the sealed cardboard package we read that the preparation was "physiologically standardized by the isolated uterus method as improved in the Mulford laboratories."

Sample II. "Solution hypophysis, Squibb," made by E. R. Squibb & Sons. "Control record" No. 11191. "A solution of the water soluble principles of the posterior lobe of the pituitary body of cattle. It has been standardized according to the method of G. B. Roth, of the U. S. Public Health Service." This product is dispensed in glass "ampuls," six in a sealed cardboard package; has the characteristic odor of pituitary extract and apparently contains no preservative agent except an excess of hydrogen ions. On the package it was stated that: "With proper keeping the contents of this package should retain its potency at least until Aug. 4, 1919."

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[1] A number of parallel tests were made with four samples of histamine. The results were so variable that no typical figures could be selected to illustrate the experiments. The height of the contractions produced by different samples at the same concentration varied, in some cases, as much as 100 per cent.

Sample III. "Pituitary liquid," made by Armour & Co.; No. 500054; 1 c. c. glass ampuls, six in a box. On the package appeared the statements: "Specially prepared and sterilized for hypodermatic injection. Free from preservatives. Standardized physiologically by the isolated uterus method and of such strength that a 1:20,000 dilution of pituitary liquid equals at least a 1:20,000,000 dilution of beta-iminazolylethylamine hydrochloride. We do not recommend the use of contents of this package after September, 1919."

Sample IV. "Pituitrin," O. obstetrical, made by Parke, Davis & Co.; 1 mil glass ampules and "each mil contains: Chloretone (chloroform derivative), 0.005 gm. (1/12 gr.)." The activity of this preparation had been "determined by physiological tests." May, 1919, was given as the expiration date.

Sample V. Pituitary extract manufactured by Eli Lilly & Co.; No. 4124–590395F. This product was marked: "Physiologically standardized. Prepared from the posterior lobes of fresh pituitary glands with acetoform (chloroform derivative) 1/2 per cent." The extract is dispensed in 1 c. c. glass ampules, one-half dozen in a sealed package. The serial number appears on the package, on the individual cardboard cylinder about each ampule, and on the ampule label. The package was marked: "If kept at a temperature from 40° to 50° F., the contents of this package should retain its potency at least until Jan. 15, 1919."

Sample VI. "Hypitin," made by the Norwich Pharmacal Co.; No. 55587. This product is furnished in sealed packages containing one-half dozen ampules. The extract has a pale amber color, traces of solid matter, and, according to the label, "each mil contains chloretone 0.005 gm." The liquid has been: "Physiologically tested. Sterilized. Prepared for hypodermic use." The use of this product is not recommended after April 1, 1919."

Sample VII. "Hypoloid, infundin," pituitary (infundibular) extract prepared by the firm of Burroughs, Wellcome & Co., London (England). Control record numbers and letters are 56681, 672, and WD2. This extract is furnished in heavy glass "phials," six in a box, each having a separate serial number. The fluid is faintly opalescent and very pale amber colored. The booklet inclosed in the sealed package contains the statement that the extract has been "standardized by its action on the isolated uterus."

II. EXPERIMENTAL PROCEDURE.

Since Fenger (1916) showed that pituitary extracts deteriorate at room temperature within a few hours when diluted with Locke solution the preparations were diluted with distilled water. Using

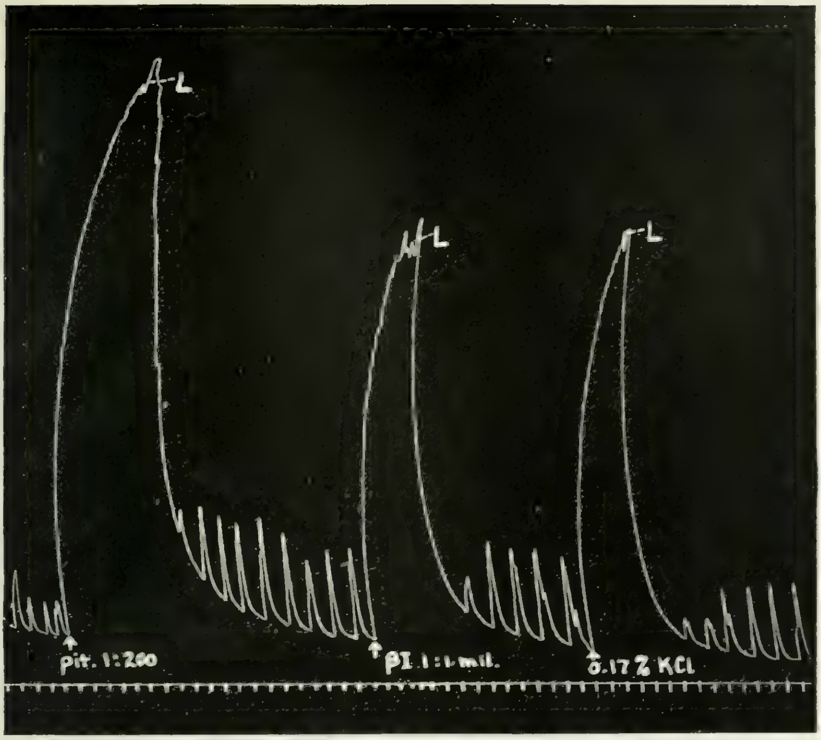

Fig. 5.—Tracing showing the qualitative similarity in the effects of histamine and KCl. L=Locke; time=minutes.

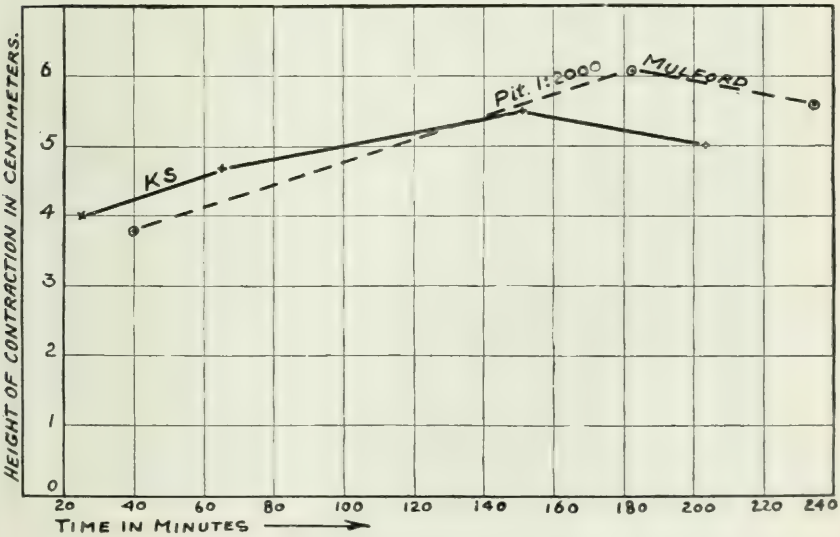

Fig. 6.—The progressive sensitivity of an isolated uterus preparation in the course of four hours. At the beginning of the experiment the sensitivity is low both to the potassium standard and to the pituitary extract. A maximum sensitivity is attained in about three hours, after which both curves fall.

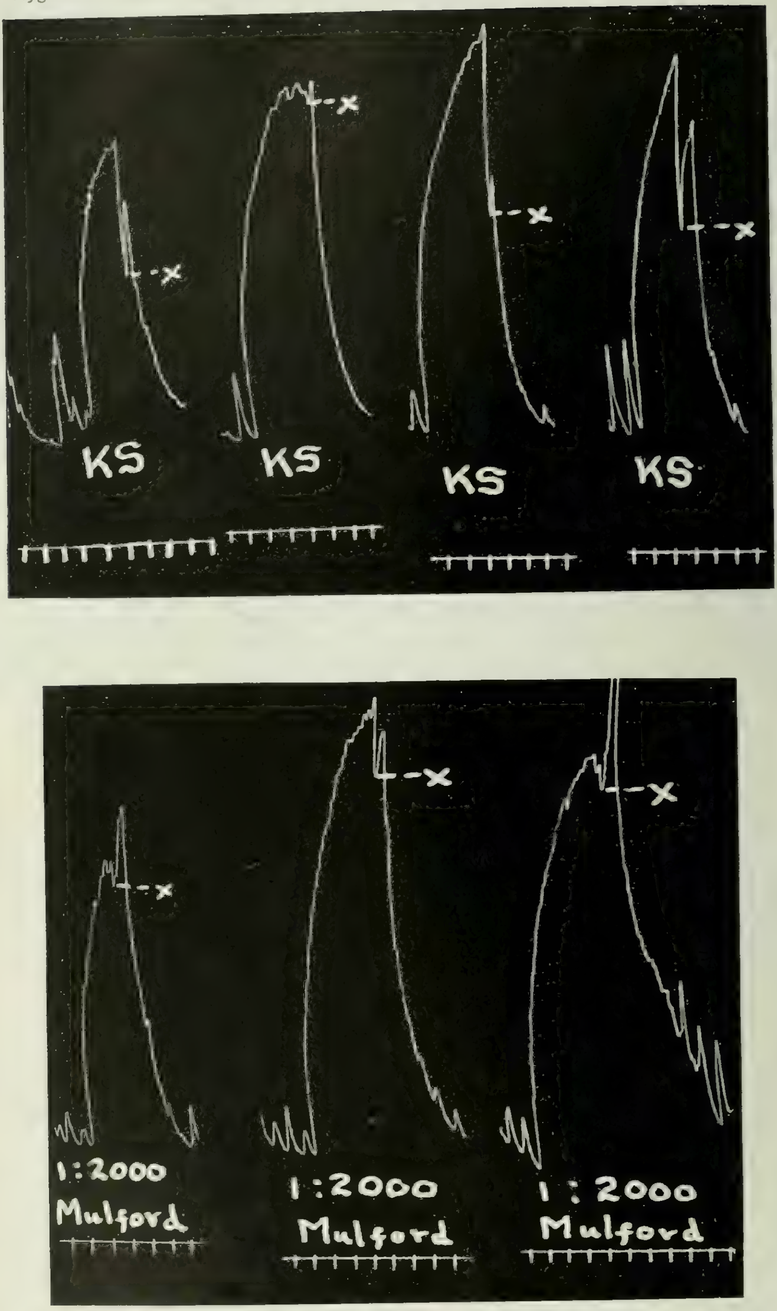

Fig. 7.—The tracings from which the sensitivity curves in figure 6 were plotted. The intervals between contractions may be read off directly from figure 6. KS=potassium standard; x=Locke; time=minutes.

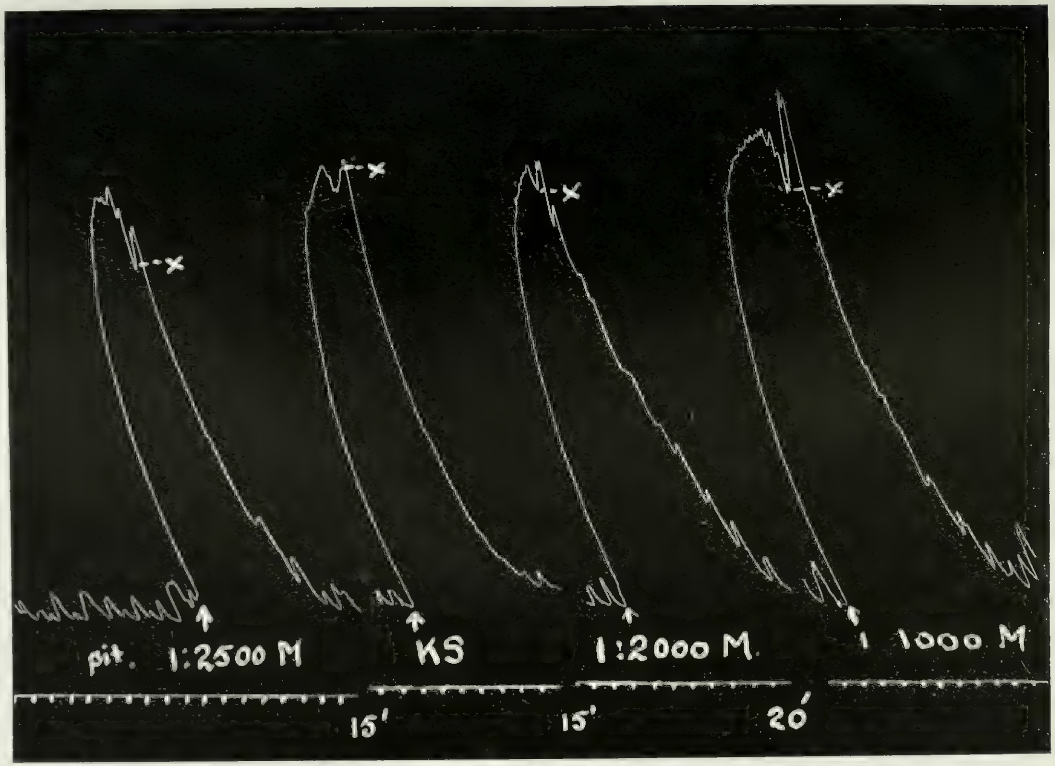

Fig. 8.—A typical standardization experiment showing the relative effects of potassium standard (KS) and three concentrations of Sample I. Time=minutes; x=Locke.

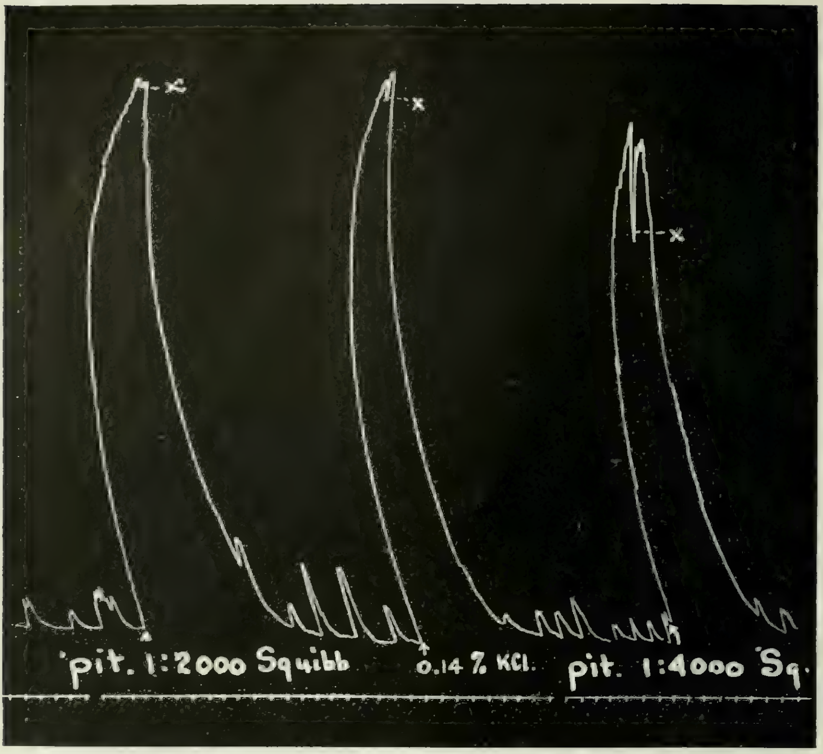

Fig. 9.—Tracing showing the effects of Sample II. Notation as before.

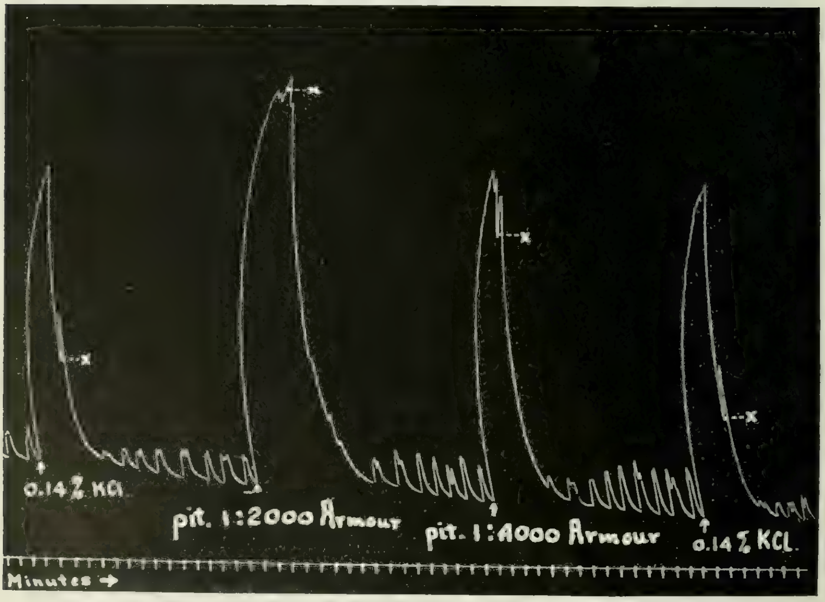

Fig. 10.—The effects of Sample III.

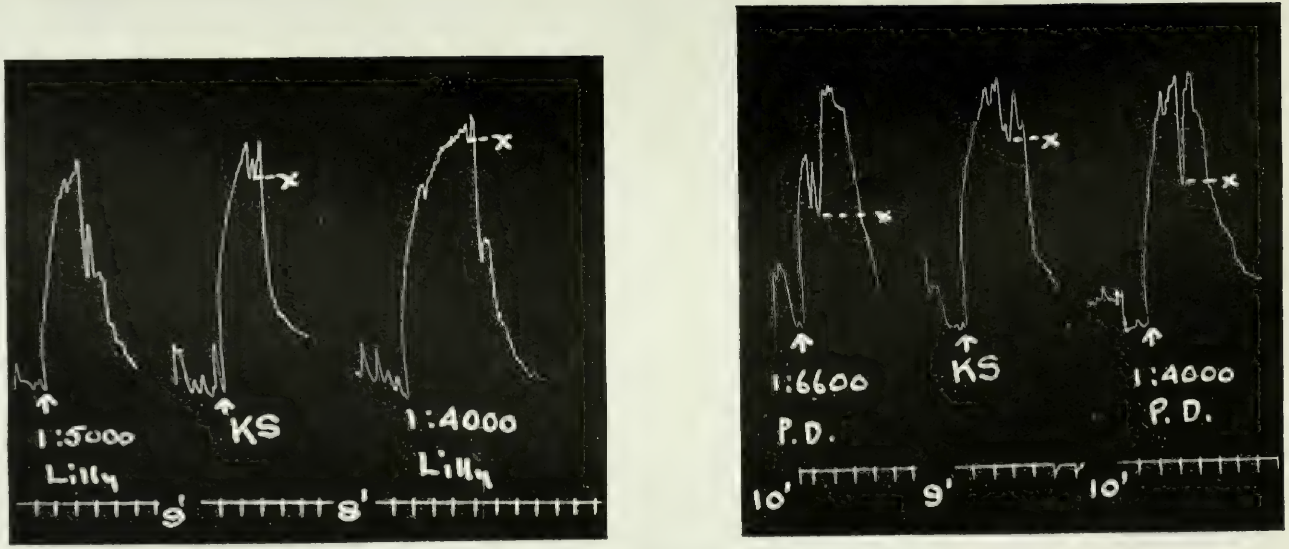

Fig. 11.—Effects of Samples IV and V on the same preparation.

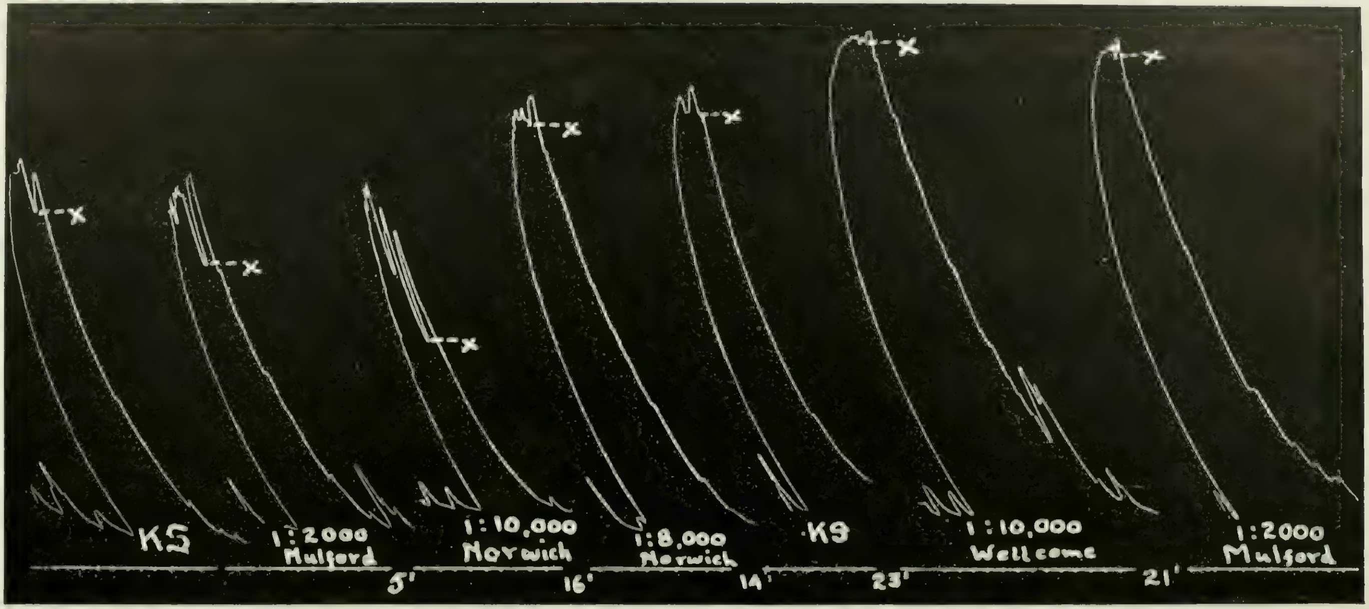

Fig. 12.—Tracing showing the relative effects of the potassium standard, Samples I, VI, and VII, on the same preparation. Notation as in previous figures.

a carefully cleaned and dried pipette, we always removed 0.5 c. c. of the ampule contents to a 10 c. c. glass-stoppered graduate and diluted to 10 c. c. with glass-distilled water. This gave a solution of 1:20. All the dilutions were made with the same pipette, using the interval 0.3 to 0.8 c. c. Requisite volumes of this 1:20 aqueous solution were then added to the Locke solution in the suspension chamber, diluting the total concentration of the pituitary extract in a range of 1:1,000 to 1:10,000. Since slight errors occasionally appeared in measuring small volumes from the one CC pipette, a dilution curve-total concentration of pituitary extract was plotted against the volume of pituitary extract added to the suspension chamber and the corrected total concentration read off directly from the graph. This precaution is recommended especially in cases where the strength of an unknown solution of pituitary extract is being determined.

III. EXPERIMENTAL RESULTS.

In studying the action of the potassium standard solution upon isolated uterus strips it became evident that the sensitivity of the uterine preparations varied in a constant fashion. The sensitivity of the strips was always low at the beginning of an experiment, increased in the course of the second and third hour to a maximum value, and then rapidly decreased, the preparation normally becoming useless after four or five hours.

Figure 6 shows the course of the variations in sensitivity of a single uterus strip both to the potassium standard and to a solution of pituitary extract, 1:2,000 Mulford (sample I). It is evident that the uterus shows a gradual increase in general sensitivity, responding with an increased contraction both to the potassium standard and to the pituitary solution. After attaining a maximum height, the contractions again become smaller, both sensitivity curves dropping in approximately parallel fashion.

The actual tracings made by the uterus strip from which the data plotted in figure 6 were obtained are shown in figure 7. The figure is self-explanatory.

A study of figures 6 and 7 shows that sample I, in concentrations of 1:2,000 is about equal to the potassium standard. This conclusion is further corroborated in figure 8, which shows additional responses both higher and lower than the potassium standard.

Repeated experiments with sample II showed that it had about the same strength as sample I. Figure 9 represents a characteristic tracing indicating the effects of sample II (Squibb). The potassium standard corresponds approximately to a concentration of 1:2,000. The Squibb preparation is the only one of the seven samples tested which had been standardized by the makers strictly according to Roth's recommendation. The Mulford product (sample I) is the only extract which the manufacturers claim to be stronger than the U. S. P. The U. S. P. IX recommendation calls for a solution that is twice as strong (1:20,000) as that suggested by Roth (1:10,000). Since samples I and II are found to be of about equal strength, either (1) the Mulford pituitary extract had deteriorated or (2) the histamine with which it had been standardized had deteriorated. In the light of the writer's experience and the observations of Pittenger and Vanderkleed (1917) the latter alternative seems highly probable. Thus instead of being "stronger than the U. S. P.," as the makers claim, sample I appears to be only one-half as strong as the U. S. P.

Sample III (Armour) proved to be somewhat stronger than I and II. Figure 10 shows a typical tracing. In other tests the concentration of 1:4,000 fell below that of the potassium standard. We finally concluded that a concentration of 1:3,000 was approximately equal in effect to that of the potassium standard.

Samples IV and V, Parke Davis & Co. and Lilly, we found to have nearly the same strength, though both were stronger than samples I–III. Fig. 11 shows the effects of these two samples on the same uterus strip. The figure shows a concentration of 1:4,000 to produce a contraction in sample IV of about the same order as that brought about by the potassium standard. Additional experiments showed that the Lilly preparation was consistently slightly stronger than sample IV.

The last two samples, Nos. VI and VII (Norwich and Burroughs and Wellcome Co.), were by far the most powerful commercial products tested. A series of tests were carried out upon separate uterus strips and finally compared both samples with the Mulford preparation (sample I). The result of one of the latter experiments is shown in figure 12. The Mulford product again appeared equal to the potassium standard (cf. figs. 7 and 8) in a concentration of 1:2,000, while the Norwich extract was from four to five times as potent. The Wellcome sample likewise produced a very powerful action on the uterus strips being about five times as strong as the Mulford preparation.

Roth made a comparative study of the pituitary products of six manufacturers. He reported a single experiment for each sample and controlled his work by standardizing five of his samples against the sixth, finding a satisfactory agreement by both methods. Roth distinguished between samples having 3.5 and 4 times unit strength. From our own experience we believe that differences in strength of the order of 25 per cent are of doubtful value and significance.

The following table summarizes the investigation of the seven commercial samples described above:

TABLE I.

Sample No.	Labeled.	Activity.	Acidity as P_h.
I	Pituitary extract Mulford	1	4
II	Solution hypophysis Squibb	1	4.2–4.3
III	Pituitary liquid Armour	1.5	4–4.8
IV	Pituitrin, Parke, Davis & Co	2	4.1
V	Pituitary extract, Lilly	2–2.5	6
VI	Hypitin, Norwich Pharm. Co	4–5	5–5.2
VII	Infundin, Bufroughs & Wellcome Co	5	4.4–4.9

IV. HYDROGEN ION CONCENTRATION OF COMMERCIAL PREPARATIONS.

The acidity values, represented in the above table by the logarithm of the reciprocal of the hydrogen ion concentration P_H, were determined by comparing 2 cubic centimeters of the pituitary extracts in aqueous solutions of 1:20 with 2 cubic centimeters of standard phosphate mixtures, both solutions containing equal quantities of various indicators. The variations in the P_H values are due to the fact that three indicators were used which gave different results. We used Congo red, methyl red, and tetra-bromo-phenol-sulfonphthalein. The latter frequently showed a color which was out of series, suggesting that the pituitary extract had probably produced a change in the molecular structure of the indicator, while sample II produced a slight precipitation. The Congo red was precipitated by samples I and VII. From the results given in the above table, it is clear, however (1), that all the pituitary preparations were acid and (2) that the acidity values given in the table are only approximations.

Adams (1917) has recently shown that rapid thermal decomposition of the oxytocic principle occurs in pituitary extracts at 100° C. when the acidity is of the order $P_H=5$. The oxytocic principle becomes thermo stable, however, when the hydrogen ion concentration is increased to the order of $P_H=3$.

A study of the P_H values found in the case of the seven commercial pituitary samples shows that what appears by the indicator method to be the most acid sample (No. I, Mulford) has the least physiological action. The fact that the Mulford preparation is an aluminum compound of the active principle of the posterior lobe suggests that the metallic component may influence the normal turning point of the indicator, as has been shown in the case of other trivalent cations (Hoeber and Spaeth, 1914).

The variations of physiological activity in the commercial samples of pituitary extract show no correlation with the hydrogen ion concentrations. From the observations of Adams l. c. it seems highly probable that an increase of the acidity of pituitary preparations to the order $N\times10^{-3}$ would improve their keeping qualities without inhibiting their clinical value. Owing to the variability of the indicator method the acidity determinations should be controlled electrometrically.

V. RELATION OF THE PROPOSED POTASSIUM STANDARD TO THE HISTAMINE STANDARD.

Sample II (Squibb) was standardized according to Roth's method in terms of histamine, which presumably had a maximum physiological activity. The proposed potassium standard has an effect upon uterus strips equivalent to Squibb's pituitary solution at a concentration of 1:2,000. It therefore requires a pituitary solution five times as strong (1:2,000) to equal the effect of the potassium standard as is required to equal the histamine standard (1:10,000 according to Roth). The U. S. P. IX recommendation calls for a pituitary solution of double the strength suggested by Roth; that is, a solution of 1:20,000 is supposed to equal the histamine standard. In terms of the potassium standard this would therefore mean that a pituitary solution of 1:4,000 should produce the same effect as a Locke solution containing 0.14 per cent KCl (the potassium standard). The validity of this argument hinges upon the presumption that the histamine in the standardization of Squibb's extract produced a maximum physiological action.

In our table a pituitary concentration of 1:2,000 has been taken as unity. Samples I–III therefore fall below the U. S. P. IX requirement; samples IV and V are approximately equal to it; and samples VI and VII are more than twice as strong as the pharmacopœial recommendation.

Since in clinical practice it is a simple matter to vary dosage, it seems reasonable not to raise the strength of the U. S. P. IX requirement, but to inject, if necessary, as much as 2 cubic centimeters of pituitary solution in order to produce the required effect. The chances of disastrous results from the injection of excessively strong extracts are too great to warrant the commercial production of pituitary solutions stronger than those at present on the market.

SUMMARY AND CONCLUSIONS.

1. Several theoretical and practical objections are advanced against the use of beta-iminazolylethylamine hydrochloride as a standard for assaying pituitary extracts of unknown strength.

2. The theoretical and practical advantages of using potassium chloride to produce qualitative effects upon the isolated virgin guinea pig uterus, which are comparable to the effects of pituitary extracts, have been studied.

3. It is suggested that a Locke solution containing 0.14 per cent KCl in place of the normal 0.042 per cent KCl be employed as a

new standard for determining the physiological activity of pituitary solutions of unknown strength.

4. Seven commercial pituitary preparations have been comparatively studied. Variations between the strongest and weakest solutions have been found to be in the ratio of 5:1.

5. No positive correlation has been found between the relative acidity of the commercial preparations and their physiological activity. It is suggested that in order to maintain their physiological potency commercial pituitary preparations should contain hydrogen ion in a concentration of the order $N \times 10^{-3}$ ($P_H = 3$).

6. In order to make the proposed new standard conform as far as possible with the present U. S. P. IX recommendation, it is suggested that the effect of commercial pituitary extracts in a concentration of 1:4,000 should be equal to the effect of the potassium standard solution when the isolated uterus of a virgin guinea pig is used as a test organ.

EXPLANATIONS OF FIGURES.

Figure 1. Graph showing the method of obtaining the potassium standard solution of 0.14 per cent KCl in Locke from Locke solutions of different potassium content. A 2 per cent concentration of KCl was found most practicable.

Figure 2. Tracing showing the effects of potassium chloride in doses two, three, four, and five times normal. Time=minutes; L=Locke.

Figure 3. Tracing showing the effects of a prolonged action of the potassium standard solution. Time=minutes; ×=Locke.

Figure 4. The comparative effects of four samples of KCl at a concentration of 0.14 per cent in Locke. L=Locke; time=minutes; other letters refer to names of the manufacturers.

Figure 5. Tracing showing the qualitative similarity in the effects of histamine and KCl. L=Locke; time=minutes.

Figure 6. The progressive sensitivity of an isolated uterus preparation in the course of four hours. At the beginning of the experiment the sensitivity is low both to the potassium standard and to the pituitary extract. A maximum sensitivity is attained in about three hours, after which both curves fall.

Figure 7. The tracings from which the sensitivity curves in figure 6 were plotted. The intervals between contractions may be read off directly from figure 6. KS=potassium standard; ×=Locke; time=minutes.

Figure 8. A typical standardization experiment showing the relative effects of potassium standard (KS) and three concentrations of sample I. Time=minutes; ×=Locke.

Figure 9. Tracing showing the effects of sample II. Notation as before.

Figure 10. The effects of sample III.

Figure 11. Effects of samples IV and V on the same preparation.

Figure 12. Tracing showing the relative effects of the potassium standard, samples I, VI, and VII, on the same preparation. Notation as in previous figures.

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TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

Hygiene

HYGIENIC LABORATORY—BULLETIN No. 116

JANUARY, 1920

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By CARL VOEGTLIN, M. H. NEILL, and ANDREW HUNTER

II. THE CHEMICAL COMPOSITION OF THE BLOOD OF PELLAGRINS

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I. THE INFLUENCE OF VITAMINES ON THE COURSE OF PELLAGRA.

By Carl Voegtlin, *Professor of Pharmacology, United States Public Health Service;* M. H. Neill, *Passed Assistant Surgeon, United States Public Health Service;* and Andrew Hunter, *Professor of Biochemistry, University of Toronto, formerly Biochemist, United States Public Health Service.*

INTRODUCTION.

Among recent hypotheses as to the cause of pellagra, one of the most attractive is that which attributes this disease, like beriberi, to the absence from the diet of certain essential accessory foods or "vitamines." This hypothesis was first proposed by Casimir Funk (1913), who was led to it by the many analogies existing between beri beri and pellagra and who considered it to be supported by observations of Nightingale (1914) upon a South African epidemic of so-called Zeism. According to Funk (1914), pellagra appears in a community as a result of the substitution for undermilled corn of a highly milled product, a product in which the vitamines resident in the germ and aleuron layers have been removed. It is impossible to maintain this conception in regard to the occurrence of pellagra in the United States in this original form, as many facts point to the corn meal as being only one of the factors involved in this question. (See Grimm, 1913; Goldberger, Waring, and Willets, 1915; Voegtlin, Sullivan, and Myers, 1916.) Nevertheless, it seems that the customary diet in regions where pellagra is endemic may be more or less deficient in some essential dietary constituents, a proper supply of which would prevent the appearance of the disease. The dietetic peculiarities of pellagrous communities call for more exact quantitative study than has yet been devoted to them, but it seems certain that one of the most prominent is a relatively infrequent indulgence in foods rich in animal protein, such as fresh meat, eggs, and milk. These last-mentioned foodstuffs are known to be relatively rich in so-called vitamines, which may shield the consumer from pellagra. This possibility is one which would seem to be capable of an experimental test. A preliminary attempt at such a test has been made upon 13 selected cases of pellagra and the results, inconclusive in some respects, yet encouraging, are here reported.

On the grounds just indicated it seemed that the vitamines giving the greatest promise of success would be those prepared from fresh foods rich in animal protein. These were accordingly the first to be made use of. Later, the writers employed Funk's vitamine fraction of yeast and rice polishings, and some less purified preparations (activated Lloyd's reagent) from autolyzed yeast.

PREPARATION AND PHYSIOLOGICAL EXAMINATION OF "VITAMINE" MATERIAL.

The vitamine preparations used in this investigation were prepared, under the direction of one of the writers (V.), by Drs. Lubs and Myers at the Hygienic Laboratory, and by Dr. Sullivan and Mr. Jones at the Pellagra Hospital in Spartanburg. The procedure for obtaining the extract from ox liver was as follows:

Extract from ox liver.—Fresh ox liver was minced in a meat chopper and dried, first on glass plates at about 40° C. in a current of air from an electric fan, and then in a vacuum desiccator. The dried material was then ground to a fine powder and extracted with 95 per cent alcohol for several days at 37° C. About two liters of alcohol were used for each kilo of dried material. One half of the alcohol was used for the first extraction, and the remainder for the second extraction. During the extraction the bottles containing the material were occasionally shaken. The alcohol was then filtered off and evaporated in vacuo at 35 to 40° C., until the residue in the flask showed a tendency to form viscous bubbles which rise in the flask. The viscous residue was then poured into a separatory funnel and extracted with about twice its volume of ether. Three distinct layers formed, upon allowing the contents of the funnel to stand for three hours. The upper layer consisted of an ethereal solution of fats and lipoids, the middle layer of a white or yellowish insoluble residue, and the lower layer of a yellowish or brown colored oil-like substance. The material contained in the lower layer will hereafter, for the sake of brevity, be designated as the "vitamine" fraction. The "vitamine" fraction was then run off into another separatory funnel, containing ether, and thoroughly agitated. After it had settled out, the oily material was run off into a dish and the ether evaporated off in vacuo. The yellow oil had a pleasant, nutty odor, and was soluble in water. Cooper (1913) states that this material is soluble in absolute alcohol, but it was found that its solubility was very slight in absolute alcohol; whereas 50 per cent alcohol dissolves it quite readily. In case a sharp separation of the various layers was not produced upon extraction with ether, as much oily material as possible was run off, the mixture of oil and solid material was run into a dish, freed from ether and the mixture extracted with a small amount of 50 per cent alcohol. After filtering the alcoholic solution was again poured into ether and the lower layer was treated as above. The various vitamine fractions were then united and the ether evaporated in vacuo.

Extract from thymus gland.—In the preparation of the vitamine fraction from the thymus gland of the hog, the following changes were found to be necessary in the above method. Thymus glands could not be ground and dried in the usual way on account of the difficulty presented by the large amount of connective tissue. The glands were, therefore, cut up with scissors into small pieces and allowed to stand for several days in 95 per cent alcohol; for 6,000 grams of glands six liters of alcohol were used. The alcoholic extract was then filtered off, the residue placed in a filter press and the remainder of the liquid removed in this manner. The pressed-out thymus glands were then minced in a meat chopper and again extracted with 8 liters of 95 per cent alcohol. The filtrate from the first extraction was evaporated in vacuo. On account of the tendency to foam, the filtrate was slowly dropped into the flash by means of a longnecked separatory funnel. The weight of the sirupy residue obtained from the first extraction amounted to 120 grams. The second alcoholic extract was treated in the same manner. These fractions were then extracted with ether in a separatory funnel. The lower layer, containing the vitamine fraction, was then run off and the ether evaporated in vacuo. The greater part of the vitamine is extracted by the first treatment with alcohol. The two fractions were united and weighed 181 grams.

Yeast vitamine 62.—Fifteen kilos of dried brewers' yeast were extracted on the water bath with 6 liters of 50 per cent alcohol, containing 0.3 per cent sulphuric acid. The mixture was then brought to the boiling point and kept there for 15 minutes, filtered through a large Buchner funnel and the residue on the filter washed with warm 50 per cent alcohol. The combined alcoholic filtrates were evaporated under reduced pressure to a small volume. The turbid concentrate was filtered on a Buchner funnel; the washings were practically colorless and free from acid. The combined filtrates were treated with a slight excess of neutral lead acetate in order to remove coloring matter, proteins, sulphates, phosphates, etc. The filtrate from the lead precipitate was freed from lead by means of hydrogen sulphide. The resulting solution was evaporated under reduced pressure to about 2 liters. On standing, a precipitate settled out which was mainly inorganic and was filtered off. The filtrate was treated with silver nitrate. A bulky precipitate formed, which was filtered off under pressure and was washed thoroughly with warm water. The filtrate was made alkaline to litmus by means of barium hydroxide. The resulting precipitate was filtered off, washed with water, and suspended in water containing enough dilute sulphuric acid to render the mixture slightly acid. The suspension was then freed from silver by means of hydrogen sulphide and warming on an electric plate. The silver sulphide was filtered off while hot and washed thoroughly with water. The filtrate from the silver sulphide was concentrated under reduced pressure at 40 to 50° C. to about 100 c. c. The concentrated solution was freed from sulphuric acid with barium carbonate, filtered and brought to a slight acidity with sodium carbonate. The resulting solution was treated with enough 95 per cent alcohol to make a 75 per cent alcoholic solution. This treatment threw out a precipitate; the mixture was heated to boiling and immediately filtered. The filtrate was then freed from alcohol in vacuo.

Five-tenths of a cubic centimeter of this final solution equal 0.35 gram of dried yeast.

A similar vitamine fraction was prepared from rice polishings.

Activated Lloyd's reagent.—In the preparation of this material the directions given by Seidell (1916) were followed. The preparation consists of a special fuller's earth, washed with water and treated with autolyzed yeast filtrate. After standing with the yeast filtrate for several hours the fuller's earth is filtered off with suction. The residue is washed with water and a small amount of alcohol, and dried in a vacuum desiccator over concentrated sulphuric acid.

The authors are indebted to Food Analyst Kenneth Jones for the analysis of the following vitamine preparations:

Liver extract No. 1.

Total volume 1,600 c. c.=20.3 kilos of fresh liver.
80 c. c.=approximately 1 kilo of fresh liver.

	Per cent.
Dry matter	32.68
	32.91
Average	32.80
Ash	2.678
	2.751
Average	2.714
Ash computed to dry weight	8.196
	8.361
Average	8.278

Nitrogen:

Total	3.81 per cent.
Purine	Murexide test negative.
Amino	Doubtful.

	Grams.
Sulphur—1 c. c. contains	0.00012
	.00011
	.00013
	.00014
Average	.00013

	Per cent P_2O_5.
Phosphorus	1.26
	1.23
Average	1.25

Liver extract No. 3.

Total volume 2,020 c. c. = 16.935 kilos of fresh liver.
1 c. c.=approximately 8.33 grams.

	Per cent.
Dry matter	22.840
	22.890
Average	22.860
Ash	2.470
	2.550
Average	2.510

Nitrogen:

	Per cent.
Total	2.780
	2.800
	2.830
Average	2.800
Amino	.570
	.590
Average	.580
Purine (below limit of error), less than	.006
Uric acid (below limit of error)	.006

No positive murexide test.

Sulphur: Negligible trace.

	Per cent P_2O_5.
Phosphorus	0.81
	.84
	.83
Average	.83

Liver extract No. 4.

Total volume 3,500 c. c.=35.61 kilos of fresh liver.
1 c. c.=10.17 grams of fresh liver.

	Per cent.
Dry matter	18. 490
	18. 530
Average	18. 510
Ash	2. 290
	2. 320
Average	2. 310
Nitrogen:	
Total	1. 580
	1. 610
	1. 550
Average	1. 580
Amino	.320
	. 330
Average	.330
Purine nitrogen: Murexide negative.	
Sulphur	.086
	.191
	.217
	. 115
Average	.152
	Per cent P_2O_5.
Phorphorous	0.539
	.568
Average	.554

Liver extract No. 5.

Total volume 2,280 c. c.=38 kilos of fresh liver.
60 c. c.=1 kilo of fresh liver.

	Per cent.
Dry matter	29.07
	29.13
	29.21
Average	29.14
Ash	3.91
	3.85
Average	3.88

	Per cent.
Nitrogen:	
Total	2.26
	2.21
	2.22
Average	2.23
Amino	.41
	.49
Average	.45
Purine—	
0.0014 per cent (below limit of error).	
Murexide test negative.	
Sulphur	.280
	.293
Average	.287
	Per cent P_2O_5.
Phosphorus	1.376
	1.332
Average	1.354

The ash of the various liver preparations consists mainly of phosphates, carbonates, sodium, potassium, and traces of sulphates.

Activated Lloyd's reagent No. 1.—Fifty grams Lloyd's reagent were activated by shaking with 1,000 c. c. of autolyzed yeast filtrate.

One gram of reagent contained the active vitamine from 20 c. c. yeast filtrate, or 30 to 40 grams of pressed brewer's yeast.

	Per cent.
Total nitrogen	2.34
	2.32
Average	2.33

Activated Lloyd's reagent No. 2.—Same method of preparation as above.

	Per cent.
Total nitrogen	2.32
	2.24
Average	2.28

Activated Lloyd's reagent No. 2.—Same method of preparation as above.

	Per cent.
Total nitrogen	2.38
	2.36
	2.38
Average	2.37

The activated Lloyd's reagent was tested for the presence of tryptophane, lysin, cystin, and histidine; the tests showed an absence of the first three amino acids and indicated the presence of histidine (diazo reaction, bromine reaction). The preparation contains considerable adenine.

Each type of these preparations was tested for its physiological activity on pigeons, which had developed polyneuritis as a result of an exclusive diet of polished rice. In the case of the activated Lloyd's reagent the dose necessary for the prevention of polyneuritis in pigeons fed exclusively on polished rice was also determined.

The following table illustrates the curative properties of these preparations and the equivalent of fresh material corresponding to 1 gram of the preparation. It should be emphasized that the figures under the heading of curative dose are not always the minimal dose bringing about the cure of polyneuritis in pigeons.

TABLE 1.

Source and number of preparation.	Total weight of fresh raw material used.	Total weight of finished preparation.	Equivalent of 1 gram of preparation in grams of fresh material.	Curative dose in pigeons with polyneuritis.
		Grams.	*Grams.*	*Grams.*
Liver 1a			1,000	0.06
Liver 3a	4,220	50.8	83	.3
Liver 6	6,500	68.5	102	
Liver 7	10,700	83.0	130	.1
Liver 9	4,084	47.0	87	.125
Liver 10	5,400	67.0	81	
Thymus		181.0	33	.15
Yeast vitamine 62	74,000		35	[1]5

[1] Cubic centimeters.

As regards the activated Lloyd's reagent, it may be said that this product was very effective in preventing polyneuritis and loss of body weight of pigeons on an exclusive diet of polished rice. Daily administration of 25 milligrams per bird was sufficient for this purpose.

The preparation from rice polishings (Funk's vitamine fraction) was very efficient for relieving the paralytic symptoms of polyneuritic pigeons.

The extracts from the animal tissues (liver and thymus) were of a considerably lower efficiency, but contained probably both the antineuritic substance and the fat-soluble vitamine, as it was shown by McCollum and Davis that this latter substance can be extracted from egg yolk and animal tissues by means of alcohol.

TREATMENT OF PATIENTS AND RESULTS.

The ideal method of testing the efficiency of such preparations would plainly be to administer them, unsupported by any other therapeutic measures, to patients who, while receiving a diet deficient in vitamines, had shown a steady advance in the pellagrous symptoms or at least an absence of improvement. We were able to approximate this ideal in most of the cases here reported. All of the patients with exception of cases No. 3, 4, and 11 were treated at their homes. The patient's diet was not changed in any way from that to which he had been accustomed. A notebook was furnished the patient or his relatives and the foods consumed each day were entered into this book under the constant supervision of the investigators.

Cases No. 3 and No. 4 were hospital patients and were kept on a diet resembling very closely that which the patients had consumed prior to the onset of the pellagrous attack.

Case No. 11 was also treated at the hospital, but this patient had been on a mixed diet for several months before the treatment with antineuritic vitamine was instituted.

A preliminary period of observation preceded the administration of the various preparations. Every possible precaution was taken in order to insure that the patients consumed the prescribed preparations.

The amounts of the liver and thymus preparations were selected arbitrarily. The daily dose of these for an adult was approximately the amount of extract obtained from one kilo of the fresh liver or thymus. The amounts of the yeast and rice polishings preparations were based on the effect of these preparations in relieving the paralytic symptoms of fowls suffering from avian polyneuritis induced by an exclusive diet of polished rice. The effective dose of each of these preparations was determined in this way. The amounts administered to the patients, based on body weight, were several times the doses necessary for the cure of paralysis in the fowl. If our patients were suffering from a dietary deficiency similar to that obtaining in avian polyneuritis, it seemed reasonable to expect a favorable result from the administration of these preparations, provided degenerative structural changes were not too far advanced.

In the diagnosis and treatment of these cases the writers had the valuable cooperation of Passed Asst. Surg. R. M. Grimm.

Case No. 1.

(White, male, 1½ years old.)

Past history.—Patient's mother had definite attacks of pellagra in August, 1915, and in July, 1916. No history of other cases of pellagra in family. In October, 1915, patient first exhibited cutaneous manifestations of pellagra, consisting of redness and desquamation of the skin at side of nose and on dorsal surfaces of both hands. History of duration of attack not obtainable.

Present illness.—June 1, 1916. The patient exhibited further evidences of pellagra consisting of symmetrical redness of skin covering knuckles of both hands, sore mouth, redness of tongue, and diarrhœa.

June 19. Pellagrous dermatitis has extended to cover dorsal surfaces of both hands and encircles wrists. Physical examination reveals a poorly developed and emaciated boy, but shows no other definite evidence of disease except for redness of mucous membranes of mouth and pellagrous dermatitis of hands and wrists. Patient irritable, weak, in poor general condition. Vitamine treatment. 40 c. c. yeast vitamine No. 62.

June 20. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 21. Same.

June 22. Pellagrous skin lesions as at last note. Child has a digestive disturbance; vitamine treatment omitted.

July 6. Patient has made a good recovery from his digestive disturbance. Family physician states that skin lesions began to improve about a week ago. To-day general condition seems about as when seen on June 19, previous to the vitamine treatment.

July 26. Patient's mother states that since July 6 patient has seemed as well as usual. Appetite and digestion good. Examination shows slight symmetrical areas of redness of the skin at sides of the nose. There is definite symmetrical reddening and roughening of the skin over the knuckles of both hands. Over the metatarsal bones and extending around both margins of the dorsal surface of the feet as far back as the malleoli the skin is red and thickened. Mucous membranes of the mouth normal. Knee jerks normal. Physical examination otherwise normal. In short, patient shows fresh evidences of the skin manifestations of pellagra, the third of such attacks within a twelvemonth.

August 1. General condition as last noted. Pellagrous dermatitis on hands has faded, but definite extensions of the process have occurred on the face and feet. Tongue reddened; knee jerks normal.

Patient removed from home in country and brought to Spartanburg to-day.

August 2. Vitamine treatment, liver extract No. 1, 20 c. c.

August 3. Weight, 8 kilos. Vitamine treatment, liver extract No. 1, 20 c. c.

August 4. Vitamine treatment, liver extract No. 1, 20 c. c.

August 5. Pulse and temperature have been normal; no digestive disturbances. Pellagrous skin areas on hands, feet, and face show redness at margins, indicating an extension of the lesions. Vitamine treatment, liver extract No. 1, 20 c. c.

August 6. Vitamine treatment, liver extract No. 1, 20 c. c.

August 7. Same.

August 8. Same.

August 9. Skin lesions have not extended; appear definitely less red. Weight, 8 kilos. Vitamine treatment, liver extract No. 1, 20 c. c.

August 10. Vitamine treatment, liver extract No. 1, 20 c. c.

August 11. Skin lesions continue to improve; condition of mouth now normal. Patient's strength and intelligence definitely improving. Vitamine treatment, liver extract No. 1, 20 c. c.

August 12. Vitamine treatment, liver extract No. 1, 20 c. c.

August 13. Same.

August 14. Same.

August 15. Weight, 8 kilos. Vitamine treatment, liver extract No. 1, 20 c. c.

August 17. The skin lesions have continued to heal; are now soft and smooth. New skin has appeared in the center of the lesions on hands and feet, while of the affected skin the brown, dry epidermis at the periphery alone remains. There has also been a definite gain in the general condition of the patient, as witnessed by less restlessness, more interest and intelligence, and greater strength. No gain in weight.

August 30. Patient has remained in same satisfactory condition as that observed on August 17. Skin lesions practically gone.

Diet.—Patient entirely breast-fed up to spring of 1916. The diet of the patient's mother consisted largely of wheat biscuits, corn bread, white rice, fat pork, potatoes, and some fresh vegetables. Up to August 1, 1916, the patient received, in addition to breast milk, small amounts of oatmeal, buttermilk, crackers, white rice, and wheat biscuits. All food was administered in a very irregular manner entirely depending on the whims of the patient. From August 1 to August 16, 1916, patient ingested in addition to breast milk only a few spoonfuls of rice and two or three crackers per day.

Summary and comment.—A case of pellagra in a child nursed by a pellagrous mother; patient had two recurrences of skin manifestations, in addition to the original outbreak, within a year. The first of these recurrences lasted from June 1 to about July 1. The second began at the end of July and had practically disappeared at the end of August. In regard to the treatment by liver extract No. 1, attention is called to the fact that the extension of the skin lesions was arrested on the fourth day of treatment and this was followed by a rapid clearing up of the skin. The treatment by liver extract was also accompanied by a definite improvement in the general condition of the patient, which persisted for at least two weeks after the treatment was discontinued.

Case No. 2.

(Female, colored, aged 25, married, housewife.)

Past history.—Probably had attacks of pellagra in the springs of 1914 and 1915.

Present illness.—In May, 1916, patient noted that the skin on the dorsal surfaces of hands and extensor surface of forearms turned dark and peeled off. Noticed darkening of skin of face more recently.

June 19. At present the patient has fairly well marked nervous manifestations of pellagra, namely, weakness, vertigo, diplopia, and burning of the feet. Digestive symptoms are not prominent, except that patient has three or four loose bowel movements a day. Appetite good. Physical examination shows a poorly developed and nourished colored woman, up and about the house. The skin of the face, at sides of nose extending on to the cheeks and on the forehead, shows some swelling and scaliness about the openings of the sebaceous glands. The arrangement is bilaterally symmetrical. There are also sharply bounded symmetrical areas on the lower portions of the forearms, where the skin is thickened, darkened, and desquamating. The pellagrous skin lesions do not seem acute. Tongue somewhat irregularly reddened, somewhat fissured; knee jerks equal, exaggerated. Examination otherwise not significant. Weight, 36.9 kilos.

August 2. Since June 19 patient's condition has shown no change. The same symptoms are complained of and the physical examination shows nothing new. Weight, 36.45 kilos. Vitamine treatment, 80 c. c. liver vitamine No. 1.

August 3. Vitamine treatment, 80 c. c. liver vitamine No. 1.

August 4. Same.

August 5. Same.

August 6. The patient states that she is stronger and is less dizzy. Menstruation, which has been absent for four months, has now appeared. Physical examination as regards skin lesions and otherwise shows no changes. Diarrhea continues. Vitamine treatment, 80 c. c. liver vitamine No. 1.

August 7. Vitamine treatment, 80 c. c. liver vitamine No. 1.

August 8. Same.

August 9. Patient continues to gain strength, symptoms of vertigo and diplopia have improved, but burning of feet continues. Patient can walk about out-doors a little. Has ceased to menstruate. Dead epidermis on face is desquamating. Weight, 36.45 kilos. Vitamine treatment, 80 c. c. liver vitamine No. 1.

August 10. Vitamine treatment, 80 c. c. liver vitamine No. 1.

August 11. Same.

August 12. Same.

August 13. Same.

August 14. Same.

August 15. Patient's general condition has continued to improve. Has walked half a mile without difficulty. Now practically free from dizziness, but burning of feet still continues. Patient has somewhat less diarrhœa. The skin lesions on the face have definitely improved in the past two weeks. Those on the arms show little change. Tongue still somewhat reddened. Knee jerks still exaggerated. Weight, 35.3 kilos.

August 25. Patient seems distinctly brighter and stronger. States that she feels very well except for pain in feet. No dizziness. Examination shows that skin lesions have continued to improve. Tongue now normal in appearance. Weight 36 kilos.

September 12. Patient states that she has felt well except for occasional digestive disturbances. Strength good, no dizziness. Skin now everywhere soft and smooth, tongue normal in appearance. Knee jerks definitely exaggerated.

Diet.—When patient came under observation, according to her statement, she was subsisting largely on the following diet: Biscuits, rice, gravy, hominy, and corn bread; also about four eggs a week and fat meat, pork and beans, once a week. Patient states that she does not take milk or fresh meat. During period of observation and treatment, from July 21 to August 14, the diet list kept by the patient comprised those articles mentioned above and in addition included certain green vegetables as green corn, Irish and sweet potatoes, melons, and tomatoes. Only fresh meat was chicken, once or twice a week. About the first of September the diet included some milk and fresh beef.

Summary and comment.—A case of recurrent pellagra of a subacute or chronic type. Patient's condition stationary for two weeks just prior to beginning of treatment. During period of treatment there was a definite gain in the patient's subjective sensations, relief from dizziness and diplopia. She became stronger and some tendency for the skin lesions to improve was noted. No gain in weight. This improvement progressed steadily for about three weeks after vitamine treatment was omitted, at the end of which time the skin lesions had cleared up and she was symptomatically well. From the evidence at hand it would seem probable that her diet included a greater variety of foods at the end of the period of observation than at the beginning.

CASE NO. 3.

(White, female, 28 years old, housewife.)

Past history.—First pellagrous attack in the spring of 1914.

Present illness.—August 17, 1914. Patient admitted to Pellagra Hospital showing acute symptoms of a comparatively mild type of erythema of the hands and forearms, slight sore mouth, burning sensations in feet and other parts of the body, dizziness, and depression. Patient has vertigo and staggers when walking. Physical examination reveals nothing noteworthy, except pellagrous manifestations.

October 1. Until recently there was noticed no change in her condition, but at present there are indications of an exacerbation, viz, a fresh band of erythema has appeared, and is spreading beyond the sharp outline of desquamating skin on each forearm. Two symmetrically placed erythematous spots are noticed on the inner side of the upper arm. The lips have taken on a fiery aspect, and the tongue, which has been nearly normal, developed the characteristic inflamed appearance. Patient complains of severe burning sensations all over the body and her depression has deepened.

October 12. Received daily doses of liver preparations equivalent to 1,000 grams of fresh liver from this date until October 26. During the first week of this treatment there was no improvement. In fact the erythema continued to spread; the patient remained very depressed and suffered severely from burning sensations.

October 24 (tenth day of treatment). Patient states that she feels very much better, and a definite improvement of the symptoms and cutaneous manifestations is noted.

October 27. No vitamine preparation is given.

October 28. The thymus preparation is given in daily doses, equivalent to 500 grams of fresh thymus, from this date until November 4.

October 29 (fifteenth day of treatment). The erythema has ceased to spread, and a sharp desquamating border has formed. The erythematous spots on the upper arm are no longer so red and the redness of the lips is hardly noticeable. The sides and tip of the tongue are still reddened.

November 3. The lips are normal in appearance, and the tongue is no longer unduly reddened.

November 9 (five days after the administration of the thymus preparation was stopped). Patient is no longer depressed but is cheerful. She states that she feels much better than she did a month ago (before the treatment was instituted). There is no active erythema. The skin is desquamating in large flakes. The lips and tongue appear normal. Burning sensations are less frequent and severe. The only symptom which seems to persist unaltered is the dizziness.

Diet.—The patient was kept from September 2 to November 9 on diet A (corn-vegetable diet).

TABLE 2.—*Food consumption of case No. 3 (in grams).*

Date.	Corn meal.	Hominy.	Pork fat.	Ham.	Cabbage.	Turnips.	Sweet potatoes.	Corn sirup.	Prunes.	Sugar.	Coffee.	Evaporated milk.	Vitamine administration.
1914.													
Oct. 8	87	10	50	15	75	50	100	30	....	40	240	20	
9	63	40	50	25	75	75	100	....	10	40	240	20	
10	117	25	40	25	95	25	100	....	30	30	240	20	
11	50	67	10	25	100	.....	100	....	30	40	240	20	
12	141	62	25	....	100	25	100	....	15	25	240	20	4 grams liver No. 7.
13	18	20	10	..	20	10	100	....	15	20	240	10	8 grams liver No. 7.
14	206	44	10	25	100	25	125	..	5	40	270	20	Do.
Total	682	268	195	115	565	210	725	30	105	245	1,710	130	
15	57	20	20	20	75	10	75	....	....	40	270	20	Do.
16	160	58	50	25	100	.....	150	....	....	40	270	20	Do.
17	130	34	30	20	100	5	75	....	3	20	270	20	6.5 grams liver No. 7 and 2 grams liver No. 9.
18	197	45	50	25	50	25	150	....	....	40	270	20	12 grams liver No. 9.
19	120	57	25	20	100	100	100	....	30	40	270	20	12 grams liver No. 9 and liver No. 3.
20	237	53	70	25	100	50	150	....	....	40	300	20	12 grams liver No. 3
21	130	62	20	25	100	10	75	....	....	40	300	20	12 grams liver No. 10.
Total	1,031	329	265	160	625	200	775	....	33	260	1,950	140	

TABLE 2.—*Food consumption of case No. 3 (in grams)*—Continued.

Date.	Corn meal.	Hominy.	Pork fat.	Ham.	Cabbage.	Turnips.	Sweet potatoes.	Corn sirup.	Prunes.	Sugar.	Coffee.	Evaporated milk.	Vitamine administration.
1914.													
Oct. 22	185	57	50	25	75	75	150	5	10	40	300	20	**12 grams liver No. 10.**
23	79	40	30	25	100	20	100	....	5	40	300	20	**Do.**
24	164	62	25	25	50	.....	150	....	....	40	350	20	**10 grams liver No. 6.**
25	74	67	15	25	25	.....	25	....	25	30	300	20	**Do.**
26	164	72	50	25	100	100	150	....	....	40	300	20	**Do.**
27	50	67	20	25	100	50	100	....	....	40	300	25	
28	162	67	50	25	75	75	150	....	10	40	300	20	**15 grams thymus.**
Total	878	432	240	175	525	320	825	5	50	270	2,150	145	
29	135	62	30	25	100	25	100	....	10	40	300	20	**Do.**
30	164	72	50	25	100	100	150	....	....	40	300	20	**Do.**
31	115	72	30	25	100	10	150	....	20	40	300	20	**Do.**
Nov. 1	117	62	50	....	100	25	150	....	....	40	300	20	**Do.**
2	245	72	50	25	100	.....	150	30	....	40	300	20	**Do.**
3	164	72	50	25	100	100	100	....	....	40	300	20	**Do.**
4	327	87	50	100	75	75	150	5	....	40	300	20	**10 grams thymus.**
Total	1,267	499	310	225	675	360	950	35	30	280	2,100	140	
5	136	82	50	25	100	50	150	....	....	40	300	25	
6	103	62	50	25	100	125	150	5	....	40	300	25	
7	136	50	50	25	.....	100	150	....	...	40	300	25	
8	168	72	50	25	90	85	150.	5	20	40	300	20	
9	204	54	50	50	100	50	150	....	...	40	300	20	
10	266	82	50	25	75	50	150	...	15	40	300	20	
11	150	72	50	25	100	50	150	....	10	40	300	20	
Total	1,163	474	350	200	565	510	1,050	10	45	280	2,100	155	
12	144	57	50	25	75	50	150	....	15	40	300	20	
13	136	62	30	25	200	15	100	....	....	40	300	20	
14	14	8	....	....	.....	.....	......	....	....	40	300	20	
15	.....	7	....	....	.....	.....	......	....	....	40	300	20	
16	......	34	....	20	90	10	75	....	....	40	300	20	
17	27	36	25	...	100	75	150	....	....	40	300	30	
18	......	62	25	25	50	25	150	....	10	40	300	20	
Total	321	266	130	95	515	175	625	....	25	280	2,100	150	
19	......	82	50	25	50	25	150	20	....	40	300	20	
20	55	50	50	25	50	75	150	....	....	40	300	20	
21	......	72	....	25	75	25	100	....	....	40	300	20	
22	......	82	30	25	.....	.....	150	20	30	40	300	20	

Summary and comment.—An acute case of pellagra of moderate severity. Regarding this case it may be said that the administration of the liver and thymus preparation coincided with an unmistakable improvement in the patient's condition, especially manifested by a subsidence of an acute (and apparently progressive) pellagrous inflammation of the skin of the arms and mucous membranes of the mouth. The mental condition and subjective symptoms of the patient also showed definite improvement.

CASE No. 4.

(White, female, 28 years old, housewife.)

Past history.—Patient had an attack of pellagra during summer of 1913.

Present illness.—In July of 1914, the patient developed a pellagrous dermatitis on the hands, forearms, and neck. The mouth was sore and diarrhœa was present.

September 29. Patient admitted to Pellagra Hospital. Has diarrhœa, some headache, and weakness. Appetite good. Up and about the wards. Examination shows a poorly nourished woman. Does not appear to be severely ill. The skin on the dorsal surface of hands, extensor surface of forearms extending up on to the elbows is rough, cracked, and reddened. Desquamation present. The pellagrous dermatitis also involves the upper arms, shoulders, back of neck, and upper portions of sternum. Tongue and mucous membranes of mouth are bright red. Patient's appetite is very good. Choking sensation.

October 5. The dermatitis on the arms has spread since last note, and a fresh spot of erythema has appeared on the chin. Soreness of tongue and lips has increased. The skin of corners of mouth cracked. Severe diarrhœa. Appetite diminished.

October 11. During the last week the patient's general condition has declined and the pellagrous symptoms have increased in severity to a marked extent. Patient refuses to take food. Is nauseated and confined to bed. Very weak.

October 12. Condition same as at last note. Administration of liver extract begun and continued until November 4. (See Table 3.)

October 13. Patient eats little food.

October 20. After the first week of treatment there was little change except that there was a sudden return of appetite. Patient has gained strength. The diarrhœa has diminished, and the condition of the mouth is rapidly improving. Choking sensation has entirely disappeared. Dermatitis still spreading about elbows. Dermatitis is gradually disappearing on forearms. Eats most of her ration. Sleeps fairly well. Is cheerful. Feels "blind" when arising quickly, but this not so frequent or severe during the past few days. Weight 58 kilos.

October 31. Condition of mouth has improved markedly. Tongue slightly reddened and mucosa of lips appears pink instead of red as before. Dermatitis is subsiding at all points. Bowels now act only twice daily. Walks about out of doors. Feels better than at any time since diarrhœa began, in August. Patient's general condition appears remarkably improved.

November 9. Skin of upper arm is desquamating in large flakes. That on the chest is normal. On the neck there is only one small area of roughened skin. Mouth is normal. Bowels constipated. Patient is actively moving about, and complains of nothing but an occasional spell of weakness and dizziness. Has gained in body weight.

November 13. Discharged from hospital.

Diet.—The patient was kept throughout her stay in the hospital on diet A (corn-vegetable diet).

TABLE 3.—*Food consumption of case No. 4 (in grams).*

Date.	Cabbage.	Corn meal.	Ham.	Hominy.	Corn sirup.	Pork.	Potatoes.	Prunes.	Turnip tops.	Coffee.	Evaporated milk.	Sugar.	Vitamine administration.	Body weight.
1914.														
Oct. 8	....	33	25	57	....	....	25	30	....	....	10	10	....	....
9	10	27	20	36	....	50	25	30	50	240	20	10	....	....
10	25	11	25	27	5	20	150	30	20	240	20	20	....	....
11[1]	....	....	....	....	....	....	....	....	....	....	....	....	....	....
12[1]	....	....	....	....	....	....	....	....	....	....	....	....	4 grams liver No. 7	....
13	....	125	15	43	....	10	20	15	10	240	20	40	8 grams liver No. 7	....
14	100	117	....	4	30	10	75	5	....	270	20	20	do	....
Total.	135	313	85	167	35	90	295	110	85	990	90	100	....	....

[1] Nothing eaten (nauseated).

TABLE 3.—*Food consumption of case No. 4 (in grams)*—Continued.

Date.	Cabbage.	Corn meal.	Ham.	Hominy.	Corn sirup.	Pork.	Potatoes.	Prunes.	Turnip tops.	Coffee.	Evaporated milk.	Sugar.	Vitamine administration.	Body weight.
1914.														
Oct. 15	5	35	25	52	....	10	15	30	5	270	20	40	8 grams liver No. 7	......
16	25	57	25	2	20	....	50	10	....	270	20	40	do.	......
17	25	87	25	31	....	15	30	30	5	270	20	40	12 grams liver No. 9	......
18	50	250	15	....	30	50	150	....	75	270	20	40	do.	......
19	100	44	25	3	5	25	50	30	10	270	20	40	12 grams liver No. 93	[1] 130
20	75	95	25	46	30	50	25	25	10	300	20	40	12 grams liver No. 3	......
21	75	38	....	46	5	30	10	20	....	300	20	40	12 grams liver No. 10	......
Total.	355	606	140	180	90	180	330	145	105	1,950	140	280	......	......
22	25	79	25	62	5	50	150	15	....	150	20	20	12 grams liver No. 10	......
23	....	19	25	5	20	30	10	....	....	300	20	40	do.	......
24	....	120	25	85	....	50	150	20	....	300	20	40	10 grams liver No. 6	......
25	....	74	25	5	20	30	10	25	....	300	20	30	do.	......
26	95	97	25	9	30	50	150	25	20	300	20	40	do.	......
27	100	16	25	28	10	30	15	15	10	300	25	40	......	......
28	100	127	30	42	....	50	150	20	....	300	20	40	......	[1] 125.3
Total.	320	532	180	236	85	290	635	120	30	1,950	145	250	......	......
29	100	87	25	25	20	30	100	15	10	300	20	40	15 grams thymus	......
30	200	204	25	37	20	100	25	30	10	300	20	40	do.	......
31	100	29	25	38	25	30	100	30	....	300	20	40	do.	......
Nov. 1	100	74	....	48	....	50	75	30	....	300	20	40	do.	......
2	75	234	25	62	30	50	120	30	100	300	20	20	1 gram liver No. 1a	......
3	150	74	25	57	....	50	100	5	....	300	20	40	do.	......
4	100	245	100	57	20	50	125	30	....	300	20	40	do.	......
Total.	825	947	225	324	115	360	645	160	120	2,100	140	260	......	......
5	100	35	50	40	20	50	150	25	25	300	25	40	......	......
6	100	49	25	57	20	50	150	20	5	300	25	40	......	[1] 126.7
7	150	122	25	40	20	50	150	....	....	300	25	40	......	......
8	....	177	25	46	....	50	100	25	25	300	20	40	......	......
9	150	204	50	62	20	50	100	30	....	300	20	40	......	......
10	150	300	25	82	60	50	150	....	50	200	20	40	......	......
11	75	130	35	72	20	50	.....	15	....	300	20	40	......	[1] 128.3
Total.	725	1,017	235	399	160	350	800	115	105	2,000	155	280	......	......

[1] Pounds.

Summary and comments.—An acute case of pellagra of considerable severity, especially as regards the skin lesions. In the period preceding the administration of the liver extract the pellagrous manifestations gradually became aggravated, until the patient was confined to bed, being seriously ill. The digestive and skin symptoms increased in severity and the patient completely lost her appetite. After treatment with the liver extract was instituted, the appetite returned rather rapidly and there was observed a gradual and uninterrupted improvement in her condition, chiefly affecting the digestive symptoms and the skin. The patient gained gradually in strength and was discharged very much improved. The striking increase in the food consumption following the administration of the liver extract is illustrated by Table 3.

CASE No. 5.

(Female, colored, aged 35, married, housewife.)

Past history.—No symptoms of pellagra until this summer.

Present illness.—August 8, 1916. Patient can not give details. Appetite poor. Has not menstruated for several months. Has had diarrhœa for several weeks. Examination shows an emaciated woman lying in bed. Cerebration retarded, depressed, confused, marked memory defect. Tongue and mucous membranes of mouth brick red, covered with whitish exudate. Pellagrous skin lesions consist of thickening and roughening of epidermis upon knuckles. Lesions crescentic, bilaterally symmetrical; also thickening and darkening of skin at sides of nose. Physical examination otherwise not significant.

August 17. General condition and examination about as when last noted. Treatment with rice polishings, vitamine No. 50, begun to-day. August 17 and 18 dose 20 c. c. daily; after this 40 c. c. daily until September 1.

August 21. General condition unchanged. Skin over knuckles desquamating.

August 24. General condition unchanged. Skin over both olecranon processes desquamating.

August 27. Appearance of tongue and hands unchanged.

September 1. Tongue and gums distinctly red, skin over knuckles of both hands shows bilaterally symmetrical crescentic areas where epidermis has become separated. Appetite and digestion good. Still weak. Thickening and darkening of skin at side of nose persists.

Vitamine treatment discontinued.

September 7. Condition as at last note.

Diet.—Consists largely of corn bread, wheat biscuits, sweet potatoes, rice, hominy, okra, cabbage, fat pork, and some beans; milk, butter, and beef were taken occasionally and irregularly.

Summary and comments.—A case of pellagra presenting well-marked mental symptoms. Digestive and cutaneous symptoms present, but not so pronounced. Condition showed little, if any, change during period of observation and treatment.

CASE No. 6.

(Colored, female, 24 years old, married, occupation housework.)

Past history.—Always well until present illness.

Present illness.—June 24, 1916. In April, 1916, patient noticed soreness and cracking of skin on backs of hands. Weak and dizzy. Quit work. In bed one week about June 1, suffered from vomiting and diarrhœa. Complains now of weakness, indigestion, diarrhœa, pain in feet, but walks a mile without marked fatigue. Examination shows fairly well developed and nourished woman, not actually ill, up and about the house. Intelligence above the average. Tongue slightly red along edges. Knee jerks slightly exaggerated, equal. Pellagrous skin lesions consist of bilaterally symmetrical roughening and thickening of skin at sides of nose, at the sides of the neck, and on the extensor surface of the forearms. Weight, 41.75 kilos. Physical examination otherwise not significant.

June 26. No diarrhœa, feels better than when seen last.

June 27. Treatment with daily doses of 100 c. c. of yeast vitamine No. 62 begun and continued until July 12, inclusive.

July 2. Since June 27 patient's general condition has seemed to improve somewhat, but still complains of pain in the feet. Bowels moving two or three times per day. Occasionally nauseated. Appetite good.

July 5. Weight, 42.20 kilos.

July 9. General condition shows little change; occasionally has some indigestion. No diarrhœa. Tongue normal. No extension of skin lesions. Areas involved same as when first seen.

July 12. Weight, 42.65 kilos. Appears less nervous than when seen June 24. Tongue normal. Skin lesions show little change. Knee jerks normal.

Vitamine treatment discontinued.

July 20. Complains only of pain in feet, which has been continuous. Strength good; skin lesions on arms well marked, desquamating; apparently some extension on the face. Tongue slightly reddened at margins. Knee jerks exaggerated. Weight, 42.87 kilos.

August 14. Since July 20 patient has been in Asheville, N. C. Well except for pain in legs. Tongue normal. Knee jerks normal. Skin lesions have practically disappeared.

Diet.—Diet before onset of symptoms consisted of hominy, corn bread, fat pork, fresh and dried beans, biscuits (wheat), potatoes, and one or two glasses of buttermilk daily. Since onset of present illness patient has modified her diet irregularly as digestive conditions dictated, but the same foods are still consumed. A representative menu for a day is here given:

Breakfast: Biscuits, gravy, and hominy.

Dinner: White potatoes, wheat bread, fat pork, corn bread.

Supper: White bread, butter, molasses, cold tea.

Summary and comments.—Patient first seen when recovering from an acute attack of pellagra. General condition and skin lesions slowly improved while under observation (up to July 20), during which time patient gained 1.12 kilos. Improvement continued after treatment with antineuritic vitamine was instituted.

Case No. 7.

(White, female, aged 7.)

Past history.—Very indefinite up to one year ago; previous to that time patient was said to have had several attacks of pellagra with occasional convulsions; no attack in summer of 1915.

Present illness.—In March, 1916, pellagrous dermatitis appeared. Sore mouth and diarrhœa since then, up and till about June 1. Seen June 2. Unconscious. Appeared to be in extremis.

June 10, 1916. Examination shows an emaciated child, lying in dorsal decubitus, eyes closed. Can be aroused only with difficulty. Evidently very weak. Pulse rapid, poor quality. Further examination not made except to note skin lesion on backs of hands and feet; face characteristic of pellagra. Skin lesions not acute.

June 11. During past week patient has had difficulty in swallowing. Has had one or two involuntary loose bowel movements per day. Temperature 37.2° C.[1]; pulse 138. General condition improved. Is conscious, but apparently irrational. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 12. Several bowel movements last night. General twitching of muscles of face and extremities noted by attendant. Patient stuporous. Careful physical examination reveals the following salient features: Temperature, 37° C.; pulse, 120. Tongue, red, not fissured. Gums, brick red. Knee jerks equal, slightly exaggerated. Skin on forehead, sides of neck, sides of nose, on backs of hands, around wrists, also areas on dorsal surfaces of feet and extending up shins is bronzed and desquamating. No redness. Lesion strikingly symmetrical. There are irregular, involuntary contractions of muscles of face and hands; muscles of limbs resist passive motions. Heart sounds and pulse, rapid, poor quality. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 13. Attendant states that since last note muscular twitchings have increased; three convulsions since last note. Vitamine treatment, 120 c. c. yeast vitamine No. 62.

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[1] All temperatures were taken in axilla.

June 14. Bowels not so loose. Examination shows child to be less stuporous. Conscious but irrational. Temperature, 37° C.; pulse, 100, of better quality. Mucous membranes less red. Involuntary movements of facial muscles continue. Typical athetoid movements of both hands. Resistance to passive motions of legs noted. Knee jerks very active. Double ankle clonus now present.

June 15. Patient stated to have been delirious last night. Said to have had three convulsions this morning. Bowels move once a day.

Examination: Patient seems brighter. Temperature, 38.6° C.; pulse, 100. Poor quality. Involuntary muscular movements less marked. Reflexes unchanged.

Vitamine treatment, 120 c. c. yeast vitamine No. 62.

June 16. Seems more rational. Quieter. Involuntary muscular movements nearly gone. Has diarrhœa. Delirious last night.

June 18. Temperature, 36.4° C.; pulse, 92. Condition as at last note. Skin lesions show no change. Diarrhœa continues.

June 20. Nervous symptoms continue to abate; less diarrhœa. Patient is clearer mentally. Tongue less red than when first seen. Reflexes unchanged. Bowels move once or twice a day. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 21. Condition unchanged. Temperature, 37.2° C.; pulse, 110.

June 23. Condition same as last noted, except for diarrhœa during past 24 hours. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 24. Diarrhœa continues. Restless and delirious at night. Twitching of muscles again observed.

June 26. Nervous symptoms continue; one convulsion yesterday. Epidermis over skin lesions desquamating rapidly. Temperature, 37.2° C.; pulse, 100.

June 27. Said to have had a chill yesterday, with fever. Has severe diarrhœa. Nervous symptoms abating. Temperature, 37.2° C.; pulse, 104. Appetite poor. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 28. Diarrhœa continues; eats but little; muscular twitching very slight. Temperature, 36.8° C.; pulse, 100.

June 29. Chills and convulsions last night. Diarrhœa continues. Muscular twitching slight. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

June 30. Patient nauseated. Seems weak. Prostration fairly well marked. Diarrhœa continues. Temperature, 36.8° C.; pulse, 120.

July 1. General condition improved. Bowels still loose. Temperature, 36.5° C.; pulse, 100. Except for gastrointestinal symptoms, patient's general condition has shown no marked change since June 15.

July 4. General condition continues to improve. Appetite has returned. Bowels normal. Sleeps well. Rational and quiet. Up and about ward. Skin lesions continue to clear up.

July 5. Vitamine treatment, 40 c. c. yeast vitamine No. 62.

July 6. Nauseated, marked diarrhœa. Slight convulsion last night and was delirious and excited. Temperature, 37° C.; pulse, 120. Twitching of muscles has reappeared.

July 7. Diarrhœa continues, but patient eats better to-day. Delirious at night. Not able to sit up.

July 8. Bowels normal. Muscular twitchings have disappeared. Gaining strength, but makes no effort to sit up.

July 18. Up and about the house. Skin lesions continue to clear up. Temperature, normal; pulse, 72.

September 11. It is stated that patient experienced a recurrence of skin lesions about August 1, without return of other pellagrous symptoms. Examination shows rather recent dermatitis on backs of hands and feet. Otherwise child has evidently gained much in strength and weight since last observation.

Diet.—Consisted from June 11 to July 14 of small amounts of cereal coffee (postum), wheat crackers, white bread made from highly milled flour, white rice, Irish potatoes, and gravy made from flour.

This diet is obviously extremely one-sided, being largely obtained from highly milled cereals.

Summary and comment.—Chronic recurrent pellagra in a child. Patient seemed desperately ill when first observed. Her general condition improved slowly but definitely while under observation, despite a temporary return of skin lesions. About June 12, June 24, and July 29 there was an increase of nervous and gastrointestinal symptoms.

The administration of the vitamine preparation in this case was followed by a moderate increase of nervous and gastrointestinal symptoms. On this account it was impossible to administer the preparation with sufficient regularity to reach a definite decision as to its efficiency. At first the giving of the preparation appeared to be coincident with some improvement in the patient's condition; however, at other times no such effect was noted. It will be observed that the patient's general condition continued to improve after the vitamine treatment had been discontinued.

CASE No. 8.

(White, female, aged 23, married.)

Past history.—No serious illness. Had symptoms like the present in March, 1915.

Present illness.—July 3, 1916. In March, 1916, gave birth to a child at full term, gradually getting stronger since then. About June 15 noticed redness of knuckles, which spread over backs of hands and upper forearms. Was dizzy. Had double vision. Pain in the feet. Digestive symptoms not marked. Now general symptoms less marked, but skin lesions have increased. Physical examination negative, save for some redness of tongue, exaggeration of knee jerks, and typical pellagrous dermatitis over backs of hands and wrists.

Vitamine treatment begun; given daily 30 grams activated Lloyd's reagent No. 6.

July 10. Complains of considerable pain and burning of the legs, margins of skin lesions red, indicating some spreading. Less indigestion than formerly. Less dizziness.

July 18. General condition about the same. Not able to work. Skin lesions apparently stationary. Tongue red. Knee jerks exaggerated.

Vitamine treatment discontinued; admitted to hospital.

September 13. Patient says she feels perfectly well. Looks strong. Tongue and skin normal.

Diet.—Consists of corn grits, biscuits, potatoes, dried beans, onions, corn bread, rice, and fat pork. From July 3 to 10 patient took, in addition to the above, on some days a fried egg and a glass of buttermilk.

Summary and comments.—A patient with a moderately severe attack of pellagra. During period of observation, from July 3 to July 18, general condition failed to improve and patient was admitted to a hospital. Apparently well when seen September 13.

The vitamine treatment, with massive doses of activated Lloyd's reagent over a period of two weeks, was evidently not accompanied by a noticeable improvement in the condition of the patient. The subsequent hospital treatment caused a rapid recovery. This fact emphasizes the negative results of the treatment with activated Lloyd's reagent.

Case No. 9.

(Male, white, 39 years old, cotton-mill operative.)

Past history.—Not obtained.

Present illness.—June 20, 1916. Information unsatisfactory on account of patient's mental condition. Cursory examination shows typical redness, scaling, and thickening of skin of back of right hand and wrist (old amputation of left arm at elbow). Up and about.

July 6. Pellagrous dermatitis now involves right arm and shoulder and stump of left arm. Tongue not red. Patient demented, but not violent. Does not answer questions. Sitting up.

Vitamine treatment begun; receives daily 40 grams activated Lloyd's reagent No. 2.

July 14. Since July 6 there has been definite extension of pellagrous erythema. Patient has become violent, requiring restraint.

Vitamine treatment discontinued.

July 19. Patient markedly demented; pellagrous lesions acute, involve chest and shoulders. Patient markedly tremulous.

July 20. Patient died.

Diet.—On account of patient's mental condition dietary record was not obtainable.

Summary and comments.—Acute pellagra involving nervous system and skin. During period of observation patient became gradually worse, in spite of the administration in large doses of a very active preparation of antineuritic vitamine. The cutaneous and also the mental manifestations increased in severity, the latter requiring his removal to an insane asylum.

Case No. 10.

(White, male, 43 years old, married, minister, and cotton-mill operative.)

Past history.—Relates symptoms suggestive of pellagra in summer of 1915.

Present illness.—April 20, 1916. In early April, 1916, patient noted redness of backs of hands after exposure to sun; weak; quit work; some paræsthesia. No sore mouth. No gastrointestinal symptoms. Appetite poor. States that he has lost 20 pounds in weight recently. Examination shows a pellagrous dermatitis present on both cheeks and on distal halves of the dorsa of both hands. Tongue slightly redder than normal, fissured. Skin in these areas shows an acute erythema. Knee jerks equal, apparently diminished. Some mental confusion; very talkative. Up and about house. Vitamine treatment begun; receives daily 20 grams activated Lloyd's reagent No. 1.

April 21. Mental symptoms marked.

April 24. General physical symptoms unchanged. Memory impaired. Pellagrous lesions darker in color, desquamating. Knee jerks exaggerated. Tongue redder than on April 20, fissured. Patient has a cough. Appetite stated to be good.

May 1. Sputum negative for tubercle bacilli. Mental condition more nearly normal.

May 3. General condition appears better, but patient states he is weaker. Mental condition more normal. Reflexes still exaggerated.

May 10. General condition fairly good; lesions on back of hands have desquamated completely, leaving skin smooth and soft. Reddish symmetrical areas below eyes persist. Appetite good. Tongue slightly reddened.

May 13. As at last note. Weight 45.10 kilos (normal weight stated to be 54 kilos). Mental condition shows no excitement.

May 15. Erythematous areas on cheek slightly less red. Still emaciated. Somewhat emotional. Skin of hands shows no spreading of dermatitis. Tongue condition unchanged.

May 29. Redness below eyes persists.

June 3. Symmetrical redness below eyes persists. No desquamation. Hands, over metatarsal bones and proximal phalanges, skin is thickened, red, and desquamating; around wrist, front and back, skin brown, thick, and red. Tongue reddened. Reflexes and general condition about as when seen two weeks ago.

June 8. Appetite poor. Apparently losing weight. Skin under eyes and on backs of hands red and scaling. General condition unchanged.

Vitamine treatment discontinued.

June 12. Apparently losing weight. Somewhat ataxic. Some digestive symptoms. Mental depression present. Skin over backs of hands seems to show a receding stage of a rather recent erythema.

June 20. Appetite better. Gaining weight. Skin lesions show no change. General condition perhaps improved. Reflexes not now exaggerated.

June 27. Patient seems depressed. Says he feels weak. Tongue somewhat red. Backs of hands red and scaling. Red areas under eyes increasing in size.

July 2. Patient feels better. Stronger. General condition improved. Skin lesions stationary.

July 7. General condition continues to improve. Skin lesions less red. Reflexes normal. Weight 49.5 kilos.

August 4. Patient states that he is losing weight and strength. Feels abnormal mentally. Moderate redness of backs of hands and areas under eyes. Knee jerks moderately exaggerated. General condition unchanged.

Diet.—From the diet record it is obvious that patient is not taking enough food. Eats biscuits, fat pork, white bread, canned sardines, eggs (one to three daily), canned fruit, beans, cabbage. Dislikes milk and has not taken any for years. No butter.

Summary.—Chronic pellagra with mental symptoms. During period from April 20 to June 8 (seven weeks of vitamine administration) patient's general condition did not improve and his skin lesions failed to clear up. His mental condition has been distinctly abnormal. While under observation, periods of moderate excitement have alternated with periods of depression. During period from June 8 to August 4 patient's condition showed some improvement.

CASE NO. 11.

White, male, aged 34, cotton-mill operative.

Past history.—Patient gives history of first onset of pellagra in fall of 1912. Appears to have had recurrences in spring and fall of 1913 and 1914; also an attack beginning January, 1915. Discharged from United States Pellagra Hospital June 2, 1915.

Present illness.—November 10, 1915. Readmitted to United States Pellagra Hospital. Patient states that one month ago back of hands became red, and mouth sore. Had diarrhea, became weak and dizzy. Lost weight.

Examination now shows: Tongue considerably reddened. Knee jerks equal, considerably exaggerated. Pellagrous dermatitis involves extensor surfaces of hands, forearms, and elbows. Skin in these areas is dried, inelastic, and desquamating. Physical examination otherwise not significant. Appetite good.

December 11. During last month patient has gained some strength. Skin lesions have cleared up considerably. Remains nervous and somewhat weak, but is up and about ward.

January 8, 1916. Since December 11 general condition not improved. Now complains of nervousness and dizziness. Tongue redder. In bed most of the time. No sign of recurrence of skin lesions.

February 12. Patient able to walk a mile, but is then exhausted. Symptoms continue. Shows marked tremor; otherwise no change. Tongue continues red.

March 11. Condition has remained unchanged. Since entrance patient has appeared to feel better one day and worse the next.

April 8. For past 10 days a marked change for the worse has taken place in the patient's general condition, especially as regards the nervous system. In bed most of time. Increase of dizziness, weakness, and nervousness. Appetite poor. Nauseated. Now shows definite involuntary twitching movements of skeletal muscles. Reflexes exaggerated. Shows marked disturbances of sensation of skin of arms and legs. No return of skin lesions; tongue very red. Now confined to bed.

Vitamine treatment begun; given daily 20 grams activated Lloyd's reagent No. 1 and No. 3.

April 15. Since April 8 patient feels that his symptoms have steadily improved. Observations have shown a definite continuous improvement in his general condition. Has become less tremulous. Tongue and reflexes unchanged.

April 22. General condition has continued to improve. Still weak and nervous. Still in bed.

May 2. Up in a wheel chair. Improvement in strength has continued. Less nervous. Paræsthesia diminished. Appetite improving. Tongue definitely less red. Knee jerks still exaggerated.

May 10. Since last observation patient has complained of being weak and tired. Up in wheel chair. Able to walk about. Still somewhat tremulous.

May 20. Vitamine treatment stopped.

May 22. General condition somewhat improved. Physical examination shows no change. Tongue red. Walks about in the street but has weakness of arms with tremor and paræsthesia of arms and feet.

June 21. Patient now up and about all day. Walks 3 miles a day without undue fatigue. Nervous symptoms much improved. No digestive symptoms; tongue still red.

July 24. Patient has gained steadily in strength; tongue still red, knee jerks exaggerated. Skin everywhere soft and smooth.

NOTE.—During observation, temperature and pulse have been within normal limits. The patient's body weight at time of admission (Nov. 10, 1915) was 46.67 kilos. The patient gradually gained in weight until March 18, 1916, when he reached 54.84 kilos (gain of 8.17 kilos). During the following seven weeks his weight was about 1 kilo lower. On July 22, patient weighed again 54.84 kilos.

Diet.—Patient received diet B (mixed diet) throughout the whole period of observation. From November 10, 1915, to March 31, 1916, the patient's daily food consumption was very regular and consisted in the following ration: White bread, 300 grams; butter, 45 grams; cornmeal mush, 50 grams; eggs, 100 grams; fresh beef, 100 grams; white potatoes, 150 grams; orange juice, 100 c. c.; dried prunes, 30 grams; sugar, 40 grams; milk (fresh), 1,000 c. c.; and coffee, 350 c. c. During the period of March 31 to July 24 the patient at times refused to eat the solid foods of the ration, especially the eggs.

Summary and comments.—Chronic recurrent pellagra. From November 10, 1915, to April 1, 1916, patient's condition improved very slowly. From April 1 to April 8, patient's nervous symptoms and general condition became definitely worse. This was followed by a definite period of improvement, beginning about 4 to 5 days after the vitamine treatment had been instituted. The improvement continued without interruption after the cessation of the vitamine treatment, on May 20, or without a change in diet. On July 24, 1916, the skin lesions had practically disappeared. Tongue remained red and reflexes unduly active long after general condition showed marked improvement.

Attention is particularly called to the fact that this patient consumed a diet of assumably high nutritive value, and which caused a great improvement in the condition of the vast majority of pellagrins treated at the United States Pellagra Hospital. In spite of this fact the patient's condition, after a period of marked improvement, became suddenly worse in respect to the nervous symptoms. Improvement again took place within a few days after the administration of antineuritic vitamine was begun, without a change in diet. It is obviously difficult to explain the cause of the "nervous attack" in this case, as the patient had taken, for several months, a diet which, a priori, should have prevented the aggravation of all pellagrous manifestations. It is possible that the sudden appearance of the nervous hyperexcitability may have been due not to pellagra per se, but rather to some other unrecognized complication. The fact, however, that careful clinical examination gave no indication of the presence of syphilis or other complication would not strengthen this viewpoint. On the other hand, it might be conceived that the patient's diet was adequate enough to produce a marked improvement in the pellagrous skin symptoms, but was not quite sufficient to supply enough antineuritic substance for the maintenance of the normal function of the nervous system. Under these conditions the administration of antineuritic vitamine (activated Lloyd's reagent) would have corrected this particular dietary defect. It should be borne in mind that the nervous manifestations of the pellagrous syndrome might very well be accounted for by a deficiency of the diet in antineuritic vitamine. Other dietary defects might be responsible for the gastrointestinal and cutaneous symptoms.

CASE NO. 12.

(Colored, female, 30 years old, married, housewife.)

Past history.—Patient states that she had her first attack of pellagra five years ago, and has had an attack every summer since.

Present illness.—July 14, 1916. Patient somewhat confused; unable to give details of present illness. Complains of dizziness, weakness, indigestion, and burning of arms and legs. Pellagrous skin lesions consist of bilaterally symmetrical areas on backs of hands and extensor surfaces of forearms, where the skin is roughened. Considerable desquamation, especially on backs of hands. Lesions evidently not acute. Further examination omitted. Weight, 43.87 kilos.

Vitamine treatment begun; 40 grams activated Lloyd's reagent given daily.

July 21. Symptoms unchanged. Physical examination shows a poorly nourished negress, up and about the house, not acutely ill. Tongue slightly reddened at margins. Knee jerks equal; exaggerated. Skin lesions as last noted. Physical examination otherwise negative.

July 28. Patient states she feels nervous and weak. Cried easily. Appetite and digestion good. Some pain in arms. Skin lesions desquamating. Weight, 44.32 kilos.

Vitamine treatment, 40 c. c. yeast vitamine No. 62 substituted for activated Lloyd's reagent from here on.

August 5. Patient recites symptoms as on July 28. Physical examination unchanged. Weight, 44.32 kilos.

August 8. Patient appeared to feel somewhat stronger since last note, but yesterday there were nausea and vomiting.

Vitamine treatment discontinued.

August 12. Skin lesions have continued to clear up slowly. Digestive symptoms returned. Patient feels stronger, but nervous symptoms persist.

September 13. Since August 12 patient has been seen several times. There has been a steady gain in strength. Nervous symptoms have disappeared. Knee jerks now normal. Skin of affected surfaces practically normal. Weight, 44.77 kilos.

Diet.—Patient's diet must be considered as somewhat more nutritious than that of the other cases. It consisted of white bread, biscuits, potatoes, cabbage, fruit, one or two glasses of buttermilk; occasionally an egg, steak, or fish.

Summary.—Chronic recurrent pellagra. During period of observation patient's condition changed very slowly. General condition seemed to improve very slightly up to August 12, including period of vitamine administration (25 days). During the following month patient showed definite improvement.

Case No. 13.

(Male, 47 years old, cotton-mill operative.)

Past history.—First pellagrous attack in summer of 1914. Was greatly improved by dietary treatment received at Pellagra Hospital during 1914–15. Discharged from hospital in autumn of 1915.

Present illness.—March 26, 1916. Worked during the past winter and up to present time, but getting weak and losing weight. Complains of constipation, burning and pains in the legs. Appetite said to be good. Weight, 62.8 kilos; has apparently lost weight. Tongue shows considerable redness; backs of hands and back and sides of the neck shows a mild hyperæmia.

Vitamine treatment begun; receives daily 15 grams activated Lloyd's reagent No. 1.

March 28. Walked 1.5 miles to hospital. Condition unchanged. Knee jerks, normal. Weight, 62.8 kilos.

April 2. Feels weaker; has had to quit work on account of dizziness. More burning in feet and legs. No digestive symptoms. Tongue and back of hands redder than at last noted.

April 4. Weight, 63.2 kilos. General condition unchanged. Skin on backs or hands not so red but fissured.

April 8. General condition and condition of mouth and skin about as last noted. Skin of backs of hands begins to desquamate. Knee jerks, slightly exaggerated. Slight swaying in Romberg's position. Beginning to-day, dose of activated Lloyd's reagent is increased to 20 grams.

April 11. Patient walked to hospital with more difficulty than on previous visits. Pain in back persistent. Condition as last noted. Weight, 62.8 kilos

April 18. Walks with a cane; weak and dizzy. Tongue very red. Redness of hands extending slightly. Knee jerks, exaggerated. No digestive disturbances. Weight, 62.8 kilos. Beginning to-day, dose of activated Lloyd's reagent is increased to 30 grams.

April 25. Skin, tongue, and general condition somewhat improved. Weight, 63 kilos.

May 2. Feels slightly stronger, but complains of burning in the feet. Skin of hands red, dry, and desquamating. Weight, 64.3 kilos.

May 9. Burning in feet continues. Has to sit down and rest after walking half a mile. Skin lesions unchanged. Tongue still red. Knee jerks, active.

May 16. General condition practically unchanged. Tongue redder. Lesions on backs of hands have extended up wrists since last noted. No digestive symptoms.

May 23. Weight, 63.15 kilos. General condition, tongue, and skin as when seen last.

May 30. Patient seems slightly stronger. Some diarrhœa. Skin and tongue unchanged. Knee reflexes not abnormally active.

June 6. Patient says he feels weak. Complains of burning in legs and abdomen. Some nausea. No vomiting. Tongue and skin lesions show little change. Vitamine treatment discontinued.

June 13. Weight 63.15 kilos. General condition and symptoms of burning in legs unchanged.

Diet.—Biscuits, corn bread, beans, turnip greens, mustard greens, fat pork, corn sirup. No eggs, fresh meat, or sweet milk.

Beginning with April 8 patient took one-half to one quart of buttermilk daily.

Summary and comments.—Chronic pellagra. Patient's condition changed very little during period of observation. Digestive symptoms, with exception of temporary nausea, totally absent. Cutaneous manifestations slow to improve. Pain and "burning" in legs persistent. Eleven weeks of treatment with an active antineuritic preparation (activated Lloyd's reagent) in massive doses did not produce a favorable change in the patient's condition. The patient was, later, readmitted to United States Pellagra Hospital and improved considerably on a mixed diet (diet B).

DISCUSSION OF RESULTS.

In order to interpret correctly the results obtained in the treatment of these cases the various preparations should be divided into two groups: (1) The extracts from ox liver and the thymus gland, and (2) preparations containing the antineuritic substance (in more or less pure form) from brewer's yeast or rice polishings. The former preparations were found to possess antineuritic properties when tested on polyneuritic pigeons. It is very likely that they also contained the fat-soluble vitamine in addition, as McCollum and Davis (1915) have demonstrated the presence of a fat-soluble vitamine in glandular organs such as the kidney and the testicle. Osborne and Mendel (1914) have also found that cod-liver oil is rich in this substance, which would indicate that the liver (of the codfish and presumably of other animals) is rich in the fat-soluble vitamine. For this reason the liver and the thymus preparations are probably of a more complex nature than those belonging to the second group which, as has already been stated, represents mainly the antineuritic substance together with some inactive impurities.

Liver and thymus preparations.—With reference to the cases treated with these preparations, it was noted in each case that a definite improvement in the patient's condition occurred while undergoing treatment. Considering cases Nos. 3 and 4, cases which were observed in a hospital and subjected to accurate dietary control and observation, it was noted that the patients were becoming worse before treatment with vitamine preparations was instituted, and, after a short interval, both patients improved definitely without change in the diet. Under these circumstances it seems logical to ascribe the improvement to the preparations administered.

In regard to cases No. 1 and No. 2 the results were highly suggestive of the efficiency of the liver preparations. In case No. 1, however, it was noted that the period of the attack during which the patient underwent treatment with liver preparation, was about the same as that of an untreated previous attack. Furthermore, the tendency of pellagra to clear up, apparently spontaneously, in children is to be borne in mind. In this case, however, the course of the skin lesions changed quite promptly, from a progression to a subsidence, after the administration of the liver preparation, and a definite improvement in the patient's general condition became obvious to a degree not previously observed.

In regard to case No. 3, the evidence is perhaps less clear, inasmuch as the dietary record and control were less satisfactory than in either of the other cases. Nevertheless, it was certain that the patient improved while receiving treatment, and had failed to improve during a preliminary period of observation before treatment was instituted.

Yeast and rice polishings preparations.—Cases Nos. 8, 10, 11, 12, and 13 received vitamines, as has been described above. Case No. 11 became worse while consuming a diet which greatly improves the majority of pellagrins. The addition of the Lloyd's reagent preparation to a diet containing a fair amount of vitamines was in this instance followed by improvement in the condition of the patient. With regard to the other cases, we were unable to secure an improvement in the condition of the patients by the administration of large doses of the activated Lloyd's reagent.

Cases Nos. 5, 6, and 7 were treated with liquid preparations obtained from yeast and rice polishings. In cases Nos. 5 and 6 the preparations were taken regularly and in large amounts, without any perceptible influence on the course of the disease. The results in case No. 7 were considered inconclusive.

SUMMARY.

1. Protein-free extracts were obtained from liver and thymus gland and also from yeast and rice polishings. The preparations obtained from liver and thymus in all probability contained a greater variety of accessory substances than did the preparations obtained from yeast and rice polishings. We have shown that the liver and thymus preparations were poorer in antineuritic substances than were the preparations made from yeast and rice polishings.

2. The preparations made from yeast and rice polishings were highly efficient for the prevention of avian polyneuritis. They were also found to be very potent in relieving polyneuritic symptoms in birds fed on an exclusive diet of polished rice.

3. The administration to cases of pellagra of these preparations made from yeast and rice polishings over a considerable period of time and in large amounts (based on the curative dose for fowls) in general failed to modify the course of the disease. Case No. 11 is possibly an exception to this observation.

4. The administration of the liver and thymus preparations to pellagrins was followed by an improvement in their condition apparently comparable to that produced by the consumption of a diet rich in fresh animal proteins.

CONCLUSIONS.

The evidence presented clearly indicates that the dietary defect responsible for pellagra is distinctly (qualitatively) different from and perhaps more complex than the one causing fowl polyneuritis and human beri beri.

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II. THE CHEMICAL COMPOSITION OF THE BLOOD OF PELLAGRINS.

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In view of the important contributions to our knowledge of certain diseases of metabolism—notably diabetes and gout—yielded by chemical investigations of the blood, and the aid of such studies to the clinician in establishing the prognosis of the case at hand, it seemed of importance to ascertain whether or not there exist any abnormalities in the chemical composition of the blood of pellagrins. It was thought that such a study might possibly yield a means of arriving at a correct diagnosis of the disease prior to the manifestation of skin lesions.

Pellagra is occasionally accompanied by a distinct impairment of kidney function, as demonstrated by the presence of albumin and casts in the urine. Some investigators have found albuminuria in a large percentage of cases; others report this condition as very rare. The fact that a pathological kidney condition has been associated at all with pellagra seemed sufficient reason for studying the nonprotein nitrogen and the urea contents of the blood.

"From a review of the literature and from our own work," says Marie (1910), "it would seem to us that secondary anæmia is a frequent, though not constant, condition in pellagra." Recently Hopkins (1915), in reporting the blood sugar findings in various diseases, has mentioned the frequent association of a definite, though often only a slight, hyperglycæmia with anæmia. The writer[1] has found that a marked hyperglycæmia often accompanies the anæmia so frequently associated with malignancy. It was thought, therefore, that the blood might have an abnormally high content of sugar and for this reason a study of this constituent was made.

The absence of free hydrochloric acid in the gastric contents of a large percentage of pellagrins, reported by several investigators of pellagra in the past and more recently by Myers and Fine (1913), Hunter, Givens, and Lewis (1916), and Givens (1918), suggested the importance of studying the chloride content of the blood.

Associated with a disturbed chloride metabolism, if that were found, an abnormal content of calcium, magnesium, sodium, or potassium might be expected in the blood. Consequently a few analyses for these constituents were made.

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[1] Unpublished work, reported in part before the New York Academy of Medicine, 1915.

METHODS.

Nonprotein nitrogen was determined by the Folin-Farmer method, using the modification of Bock and Benedict (1915), whereby the ammonia is distilled over instead of being removed by suction. For urea nitrogen, the urease method of Marshall, as employed by Van Slyke and Cullen (1914), was used. Chlorides were determined by the method of McLean and Van Slyke (1915); and sugar was estimated by the procedure of the writer with Benedict (1915).

In the analysis of the blood for the mineral constituents—calcium, magnesium, sodium, and potassium—it was desirable to employ a method that would require only a comparatively small amount of blood and one by which all of these elements could be determined in a single sample, thus again conserving the blood. Nothing original is claimed for the methods used; but in the hope that the procedure adopted may be of value to others, it is given in detail.

About 50 grams of blood were ashed by the acid mixture method of Neumann (1903, 1905). After driving off practically all of the sulphuric acid, by heating cautiously over a low flame or in a sand bath, the residue was treated with a few drops of concentrated hydrochloric acid and transferred to a flask. The hydrochloric acid was neutralized with powdered ammonium carbonate; then hydrochloric acid was added again drop by drop until a clear solution was obtained.

To remove the iron, 5 c. c. of a 20 per cent solution of ammonium actetate were added, the contents of the flask were heated to boiling, and the basic ferric acetate was filtered off and washed with 0.5 per cent ammonium acetate. The precipitate was redissolved in hydrochloric acid and reprecipitated as before. The second filtrate was combined with the first and used for the determination of calcium, magnesium, sodium, and potassium.

The calcium and magnesium were precipitated by McCrudden's method (1909–10, 1911–12), modified so that by adding no sodium or potassium reagents these mineral constituents could be determined in the filtrate remaining after the separation of calcium and magnesium.

The filtrate remaining after the removal of the iron was evaporated, if necessary, to 150 to 200 c. c., and made just acid with hydrochloric acid. Ten drops of concentrated hydrochloric acid, 10 c. c. of 2.5 per cent oxalic acid, and 8 c. c. of 20 per cent ammonia acetate solution were added and allowed to stand overnight.[1] The precipitate of calcium oxalate was then filtered off on a small ash-free paper, washed with 0.5 per cent ammonia exalate, dried at 100° C., and ashed.[1] The ash was dissolved in an excess of N/50 hydrochloric acid, the solution was boiled to drive off carbon dioxide, and the excess of acid was titrated back with N/50 sodium hydroxide, alizarin being used as the indicator. (See Treadwell-Hall, 1913.)

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[1] On account of the very small amount of calcium present the use of ammonium oxalate could be dispensed with. Cf. McCrudden, Biol. Chem., vol. 10, p. 197, 1911–12.

After filtering off the calcium oxalate and washing it, as described above, the magnesium in the filtrate was separated as outlined by McCrudden, i. e., the filtrate was evaporated to small volume with 20 c. c. of concentrated nitric acid (until fumes of nitric oxide no longer came off) and then twice to dryness after adding 5 c. c. portions of concentrated hydrochloric acid. The residue was taken up in 80 c. c. of water; 1 c. c. of 5 per cent phosphoric acid was added; the solution was neutralized with ammonium hydroxide; and then ammonium hydroxide was added until the solution contained one-fourth of its bulk of dilute ammonium hydroxide (sp. gr. 0.96). After standing overnight the precipitated ammonium magnesium phosphate was filtered off and washed with a special ammoniacal-alcohol-water solution. (See McCrudden, 1909–10.)

The precipitate of ammonium magnesium phosphate was dissolved in dilute hydrochloric acid; and after adding 1 c. c. of 5 per cent sodium citrate solution. (See McCrudden, 1909–10.) The ammonium magnesium phosphate was reprecipitated by again making the solution contain one-fourth its bulk of dilute ammonia (sp. gr. 0.96). After standing over night the precipitate was filtered off and washed thoroughly as before; then the magnesium was estimated indirectly by determining the phosphorus according to Neumann's method (1902), using N/14 sodium hydroxide and N/14 hydrochloric acid for the titration.

The filtrate and washings from the first ammonium magnesium phosphate precipitation were transferred to a 500 c. c. volumetric flask; 10 per cent barium chloride was added in excess; and then, without filtering, sufficient 10 per cent ammonium carbonate solution was added to precipitate the excess of barium. The contents of the flask were then made up to 500 c. c. and filtered; the filtrate was treated with 1 or 2 drops of 5 per cent sulphuric acid (to precipitate any barium still in solution), was allowed to stand over night, and was filtered through a dry Gooch.

An aliquot of 400 c. c of this clear filtrate was evaporated to a small volume, and finally in platinum to dryness, on a water bath. After drying at 100° and 135° C. in an oven the dish was heated gently over a direct flame to decompose the ammonium salts. The residue was taken up in water and made up to a definite volume.

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[1] It is not necessary to ash so as to obtain all the calcium as oxide, for [illegible] carbonate can be determined equally as well as the oxide.

Any insoluble material was filtered off, and the above procedure was repeated on an aliquot of the filtrate. The final residue of sodium and potassium chlorides[1] was weighed.

The potassium was determined directly in the platinum dish by the cobalti-nitrite method of Drushel as used by Myers (1909), employing in the calculation the empirical factor (1 c. c. N/10 potassium permanganate=0.0008017 gram of K_2O) adopted by the latter.

Duplicate analyses by the above procedure showed very satisfactory agreement. Any error inherent in the method, due to the introduction of traces of sodium and potassium in the reagents used, would be slight and would not affect the result from a comparative standpoint.

DIETS USED.

For at least several days immediately preceding the taking of the blood sample, the pellagrins studied had been on a definite, prescribed diet. This consisted either (diet A) of a ration rich in vegetables and cereal foods, or (diet B) of one containing a preponderance of animal protein foods, such as milk, eggs, meat, etc. The former contained a much larger proportion of carbohydrate and was low in proteins; the latter was rich in protein. From a calorific standpoint both were adequate. That diets similar to those used were sufficient for maintaining nitrogen equilibrium has been shown by Hunter, Givens, and Lewis (1916).

The composition of the two dietaries follows:

TABLE 1.—*Composition of two dietaries used.*

	Diet A.	Diet B.
	Grams.	*Grams.*
Bread (wheat), about	300	200–300
Butter	30	45
Cabbage	100	
Corn meal	50	50
Eggs (2), about		100
Ham (lean, boiled)	25	
Hominy	75	
Meat (beef or oxheart)		100
Corn sirup	30	
Milk (fresh)		[1] 1,100
Milk (boiled, in coffee)	[1] 40	
Orange juice		[1] 100
Potatoes (sweet or Irish)	150	150
Prunes (without seeds, dried)	30	30
Turnip tops	100	
Coffee	[1] 300	[1] 300
Sugar	40	40
Pork fat	50	

[1] Cubic centimeters.

Unless otherwise noted, the blood samples were taken early in the morning before breakfast; i. e. no food had been eaten since supper of the previous day.

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[1] Any sulphates present have been converted into chlorides in the fuming off of the large amount of ammonium chloride.

DISCUSSION OF RESULTS.

In Tables 2 and 3 are assembled the results of the analyses of the blood for the various constituents mentioned above. Table 2 contains the data from the patients on diet A; Table 3, those from patients on diet B. This arrangement of the report is made for the sake of convenience in discussing the results. Table 4 (p. 43) gives a comparison of the chloride content of the blood with the free hydrocloric acid of the gastric contents taken after a test breakfast, which was given immediately after procuring the blood sample.

A comparison of Tables 2 and 3 shows no constant difference in the blood of the pellagrins studied that might be attributable to the diets used, except in the total nonprotein nitrogen and urea content. With few exceptions the figures are much lower for the diet A patients than for those on diet B. In fact, the blood of the patients on diet A shows a lower total nonprotein nitrogen and urea content than the normal reported in the literature. It is of considerable interest, although by no means surprising, that, with the long-continued use of a diet low in protein, the urea, and thus the total nonprotein nitrogen content of the blood, should be comparatively low. With a limited nitrogen intake the level of urea in the blood, and a priori in the tissues (Marshall and Davis, 1914), is evidently lowered.

Patient No. 73 (Table 2) shows in one analysis relatively high figures for total nonprotein nitrogen and urea (i. e., compared with the others on diet A). This patient had been on diet A only five days prior to the taking of the blood sample, and it seems probable that the level of urea had not yet had time to be affected by the new diet. That this explanation is valid is supported by the fact that a second analysis, two weeks after the first, gave results characteristic for diet A patients. Similarly, the low figures for urea and nonprotein nitrogen found for patient No. 63 (Table 3) may be explained by the supposition that not enough time had elapsed since this patient was put on diet B for the previous low level (due to the patient having eaten a ration similar to diet A in his home) to be displaced by a typical diet B level.

Patient No. 1, who had been for months on diet A, shows (Table 2) a constant normal level for urea and total nonprotein nitrogen. This may be explained by the supposition that she had a slightly disturbed kidney function, as evidenced by the relatively high chloride, calcium, and sodium figures found. The high chloride, calcium, and sodium findings with patient No. 59 (Table 3) are not easily explained.

With the exceptions mentioned above, no notable difference in the blood of the two groups, from a chemical standpoint, were found. For this reason it seems unnecessary to discuss the clinical condition of the patients. The cases studied were of various stages of the disease and some were of the most severe type.

A glance at Table 4 shows that, although in several cases there was a complete absence of free hydrochloric acid in the gastric contents, this condition was not accompanied by an abnormal blood chloride content.

SUMMARY.

The blood of a number of pellagrins was investigated from a chemical standpoint. Quantitative determinations of total nonprotein nitrogen, urea, sugar, chlorides, sodium, potassium, calcium, and magnesium were made.

Low total nonprotein nitrogen and urea figures were found for one group of patients and are attributable to the comparatively low protein content of their diet. With these exceptions no variation from the normal was found.

TABLE 2.—*Chemical analyses of blood of pellagrins on diet A.*

Patient's number, sex, and date diet A begun.	Date blood taken (1915).	Total non-protein nitrogen.	Urea nitrogen.	Sugar.	Chlorides as NaCl.	Calcium as CaO.	Magnesium as MgO.	Sodium as NaCl.	Potassium as KCl.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1. Female; Aug. 17, 1914	Mar. 26	0.026	0.010	0.098	0.540	....	....	....	....
	Apr. 7	.026	.010	.092	.550	....	....	....	....
	Apr. 20	.022	.010	....	.555	0.0130	0.0064	0.560	0.293
51. Female; Feb. 11, 1915	Mar. 9	....	....	.104	....	....	....	....	....
	Mar. 23	.019	....	.097	.507	....	....	....	....
	Apr. 6	.019	.007	.102	.510	....	....	....	....
	Apr. 21	.019	.008	....	.509	.0089	.0059	.514	.303
	May 17	....	....	.107	....	....	....	....	....
52. Male; Feb. 15, 1915	Mar. 9	....	....	.100	....	....	....	....	....
	Mar. 23	.025	.008	.108	.453	....	....	....	....
	Apr. 6	.020	.008	.102	.468	....	....	....	....
57. Male; Feb. 26, 1915	Mar. 10	....	....	.104	.483	....	....	....	....
	Mar. 24	.025	.009	.101	.501	....	....	....	....
	Apr. 6	....	....	.107	.495	....	....	....	....
60. Female; Mar. 3, 1915	Mar. 10	....	....	.097	.506	....	....	....	....
	Mar. 23	.020	.007	.093	.514	....	....	....	....
	Apr. 7	.020	.008	.091	.500	....	....	....	....
66. Male; Mar. 21, 1915	Mar. 26	.022	.008	.098	.450	....	....	....	....
	Apr. 6	.022	.008	.113	.490	....	....	....	....
	Apr. 20	.022	.008	....	.488	.0079	.0066	.437	.324
69. Female; Mar. 29, 1915	Apr. 7	....	....	.099	.492	....	....	....	....
70. Male; Apr. 1, 1915	Apr. 7	....	....	.093	.507	....	....	....	....
	Apr. 21	....	....	.095	.501	.0084	.0070	.478	.302
	May 18[1]	....	....	.096	....	....	....	....	....
73. Male; Apr. 7, 1915	Apr. 12	.027	.012	.108	.468	.0069	.0069	.381	.297
	Apr. 22	.020	.008	.100	.460	.0076	.0079	.451	.301
82. Male; Apr. 20, 1915	Apr. 22	.022	.009	.099	.490	.0085	.0072	.514	.313

[1] Blood sample taken 3 hours after breakfast.

TABLE 3.—*Chemical analyses of blood of pellagrins on diet B.*

Patient's number, sex, and date diet B begun.	Date blood taken (1915).	Total non-protein nitrogen.	Urea nitrogen.	Sugar.	Chlorides.	Calcium as CaO.	Magnesium as MgO.	Sodium as NaCl.	Potassium as KCl.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
2. Male; Aug. 17, 1914	Mar. 10	0.026	....	0.100	0.537	....	....	....	....
	Mar. 25	.025	....	.101	.483	....	....	....	....
9. Female; Aug. 21, 1914	Mar. 11	.027	....	.107	.513	....	....	....	....
14. Male; Aug. 25, 1914	Mar. 11	.026	0.012	.096	.498	....	....	....	....
	Mar. 25	.030	.010	.092	.506	....	....	....	....
	Apr. 8	.031	.012	.101	.490	0.0080	0.0060	0.514	0.303
	May 17[1]	....	....	.100	....	....	....	....	....
36. Male; Nov. 2, 1914	Mar. 11	.029	.012	.094	.493	....	....	....	....
	Mar. 25	.031	.013	....	.484	....	....	....	....
	Apr. 8	.030	.014	.096	.486	....	....	....	....
	May 17[1]	....	....	.105	....	....	....	....	....
48. Female; Mar. 2, 1915	Mar. 11	.026	....	.105	.495	....	....	....	....
	Mar. 25	.027	.009	.096	.498	....	....	....	....
	Apr. 8	.024	.010	.101	.515	....	....	....	....
	Apr. 21	.025	.011	....	.520	.0086	.0066	.499	.298
	May 17[1]	....	....	.096	....	....	....	....	....
59. Female; Feb. 26, 1915	Mar. 9	....	....	.099	....	....	....	....	....
	Mar. 26	.027	.008	.100	.538	....	....	....	....
	Apr. 8	.024	.009	.107	.542	.0110	.0049	.610	.225
	Apr. 22	.025	.008	....	.565	.0104	.0062	.577	.250
	May 17[1]	....	....	.111	....	....	....	....	....
63. Male; Mar. 15, 1915	Mar. 23	.021	.007	.106	.476	....	....	....	....
77. Male; Apr. 13, 1915	Apr. 21	.031	.015	....	.488	.0089	.0074	.439	.340
	Apr. 22	.030	.011	....	.478	.0086	.0078	.495	.344
Normal male control[2]	Apr. 22	.030	.016	....	.476	....	....	....	....

[1] Blood sample taken 3 hours after breakfast.

[2] Mixed diet.

TABLE 4.—*Comparison of chloride content of the blood with free hydrochloric acid of the gastric contents taken the same morning.*

Patient No.	Date (1915).	Blood chlorides (as NaCl).	Gastric contents (analyzed by Givens 1918). Free HCl.	Patient No.	Date (1915).	Blood chlorides (as NaCl).	Gastric contents (analyzed by Givens 1918). Free HCl.
		Per cent.	*Per cent.*			*Per cent.*	*Per cent.*
1	Mar. 26	0.540	0.255	57	Mar. 10	0.483	0.000
	Apr. 7	.550	.190		Mar. 21	.501	.000
	Apr. 20	.555	.179		Apr. 6	.495	.000
2	Mar. 10	.537	.000	59	Mar. 26	.538	.026
	Mar. 25	.483	.000		Apr. 8	.542	.000
9	Mar. 11	.513	.139		Apr. 22	.565	.000
14	..do....	.498	.026	60	Mar. 10	.506	.113
	Mar. 25	.506	.015		Mar. 23	.514	.124
	Apr. 8	.490	.026		Apr. 7	.500	.146
36	Feb. 11	.493	.000	63	Mar. 23	.476	.018
	Mar. 25	.484	.018	66	Mar. 26	.450	.336
	Apr. 8	.486	.068		Apr. 6	.490	.222
48	Mar. 11	.495	.000		Apr. 20	.488	.321
	Mar. 25	.498	.000	69	Apr. 8	.492	.000
	Apr. 8	.515	.000	70	..do....	.507	.146
	Apr. 21	.520	.000		Apr. 21	.501	.183
51	Mar. 23	.507	.044	73	Apr. 12	.468	.000
	Apr. 6	.510	.066		Apr. 22	.460	.000
	Apr. 21	.509	.091	77	Apr. 21	.488	.248
52	Mar. 23	.453	.237		Apr. 22	.478	.270
	Apr. 6	.468	.219	82	..do....	.490	.000

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III. THE AMINO ACID FRACTIONS AND HIPPURIC ACID IN THE URINE OF PELLAGRINS.

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INTRODUCTION.

Several conditions suggestive of possible metabolic alterations are characteristic of pellagra. Gastric digestion is below par in a considerable percentage of cases on account of low acidity (1)[1] and low pepsin content (2) in the secretion of the stomach; intestinal disturbances are common at one stage or other of the disease leading in some cases to profuse diarrhœa, and in others to a very obstinate type of constipation (3); the intestinal flora in the view of many students (4) of the disease is capable in itself of producing noteworthy somatic effects; the habitual diet of pellagrins is low in protein, rich in carbohydrate (5), and in this country at least contains probably a disproportionate amount of depot fat (a term which may be used to distinguish from glandular fat, the fat elaborated and stored either by plants or animals for use as a source of energy only), and finally, the most characteristic lesions (those of the skin and nervous system) are, because of their symmetrical distribution, suggestive of some specific toxin or of some specific deficiency which for some unexplained reason finds its most vulnerable point of attack in the cervical region of the spinal cord.

It has been shown that psoriasis (6) is the sequel of a perverted tendency to retain nitrogenous materials of the food, which is alleviated, if not cured, by a strict limitation of the protein intake. Whereas, however, the lesion of this disease is the result of a supranormal growth impulse on the part of the dermatogenous cells, in pellagra the skin lesion suggests a subnormal condition. The skin is often atrophic and the entire course of the erythematous exanthem is not impossibly the result of an inhibition (trophic), exercised from nerve centres in the cord, of the normal capacity of dermatogenous cells to replace the upper layers.

The fact, already well established, that a diet relatively rich in protein and containing sufficient amounts of milk, eggs, and meat leads fairly promptly to improvement in the skin lesion and other symptoms proves only that the immediate exciting agent, or deficiency, is associated with this character of the dietary. If it could be shown, for example, that a specific protein cures the disease and this could be traced to the nature of the particular amino-acids or other constituents which it supplies, the dependence would be primary.

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[1] Reference is made by number to "Bibliography," page 71.

The most complete studies of the protein metabolism of pellagrins which have yet been made have not included the determination of amino-bodies or hippuric-acid nitrogen in the urine. Meyers and Fine (8) made daily determinations of the urea, ammonia, uric acid, creatinin, and creatin fractions leaving a rest-nitrogen of from 5 to 14 per cent of the total. Making an allowance of 0.5 per cent—a liberal estimate—for purin basis, it is clearly possible that there might be in the urines of pellagrins an unusual quantity of amino-acid or hippuric acid. The work of Hunter, Givens, and Lewis (2), already accomplished in this laboratory, has demonstrated that persons suffering from pellagra have no difficulty in maintaining nitrogen balance, even on moderate amounts of protein in the food. It is not to be expected, therefore, that any great amount of amino-nitrogen, whether free or in combination, would be encountered. Nevertheless, in these fractions of the nitrogen which habitually constitute only a very small portion (3 to 5 per cent all told) of the total nitrogen, even a slight absolute increase, if constant or if definitely associated with some outstanding symptom, would be significant.

For example, imperfect gastric digestion, now thoroughly established as a feature in some 50 to 60 per cent of the cases, might be expected to lead to imperfect assimilation of protein material, and imperfectly split products might of themselves be toxic and might be eliminated in part unchanged in the urine. Some other cutaneous diseases, such as prurigo and dermatitis herpetiformis (9) have been definitely associated with impaired digestion, the severity of the attack apparently being dependent upon the amount of imperfectly digested protein absorbed from the intestinal tract and this amount bearing a definite relationship to the amount of rest nitrogen excreted in the urine. Again, the indicanuria, which is at least a frequent accompaniment of pellagra, betokens a high degree of putrefaction in the intestinal tract. Given with this condition an abundance of raw vegetable fiber, fruits or cane sugar and we have, according to Lewin (10) and others, conditions necessary in man for the production of much hippuric acid. So far as the writer can learn, hippuric acid itself does not produce any toxic effect; but a large dose (10 grams) of benzoic acid, the precursor of hippuric acid, has been known to produce a violent rash (11). Whether the constant production of the precursors of hippuric acid in the bowel over a long period of time would produce any effect on the skin or nervous system is not yet known. The question might repay experimental study along this line in connection with pellagra.

From the considerations just recited the writer decided to undertake an examination of the urines of pellagrous patients with respect to the quantitative elimination of amino-acids, peptid-bound nitrogen and hippuric acid. The subjects employed were being studied at the same time with reference to the gastric conditions by Mr. Maurice S. Givens, and with reference to certain constituents of the blood by Dr. Robert C. Lewis. It is a pleasure to acknowledge my obligations to these gentlemen for their assistance at many points in the investigation, and to Drs. Carl Voegtlin and R. M. Grimm for their cooperation at every stage of the work.

PHYSIOLOGICAL LIMITS OF THESE URINARY CONSTITUENTS.

1. *Monoamino acids.*—In the normal course of events, the digestive split products of the proteins in the food enter the blood stream through the intestinal wall and very soon suffer one of two fates; either they are availed of very promptly for protein synthesis in needy tissues, or they are deaminated and used as a source of energy, the nitrogenous moiety being reduced to urea and eliminated from the body. It is now well established through the work of Hollweg and Meyer (12), Delaunay (13), Folin and Denis (14), Van Slyke and Meyer (15), Constantino (16), Wishart (17), and György and Zunz (18), that the amount of amino-acid nitrogen (determined as such or as residual nonprotein nitrogen) increases in the blood following the ingestion of either pure amino-acids or meat. The absolute amount of this increase is so small that, for a long time, it escaped attention altogether (19), but with the more delicate methods now in use it is seen that the relative increase at full tide of digestion may be 100 per cent or more. Soon after a meal of meat the amino-acids increase also in certain tissues, particularly the liver, but they disappear again very rapidly. In the muscles, in fact, they seem to disappear under some circumstances as rapidly as they enter; for while the residual nonprotein nitrogen does not exhibit any appreciable rise after a large meal of meat, the urea nitrogen does (17). The correct inference from these observations probably is that muscles as well as liver (20) can take part in the destruction of amino-acids (exogenous) and the consequent formation of urea, but that the liver is the chief seat of this change (21). Since the high percentage of ammonia in the portal blood (22) can no longer be regarded as proof that the amino-acids are deaminated in the intestinal wall, we may now safely infer that the fate of excess protein, as of excess carbohydrate, is determined very largely by the liver.

At all events, it is clear that the final split products of a protein meal do not remain long in the blood. Even if no tissue exhibited any special avidity for them, they could not longer be kept in circulation: for, having a relatively high diffusion coefficient, they would pass the kidney readily, just as they pass the intestinal wall. The avidity of some tissue or tissues is, however, very great. Van Slyke and Meyer (15) report that out of 12 grams of d–l alanin injected rapidly into the femoral vein of a dog more than 10 grams were removed by the tissues within half an hour; 1½ grams were excreted by the kidney; and less than half a gram remained in the circulation. The rate of entrance into the circulation here was many times that of ordinary intestinal absorption; consequently, the concentration in the blood reaching the kidney was probably much above the level attained after the richest protein meal. For this reason, the proportion excreted was much above the normal rate. Dobrowolskaja (23) has reported recently an increase in the amino nitrogen of the urine, after feeding dogs with milk and bread to which glycocoll, alanin, and glutamic acid were added in 10-gram quantities. In a second dog, however, there was an increase with alanin only. This corresponds to the experience of Lusk (24), who found that while the nitrogen of 20 grams of glycocoll is completely converted to urea by the phloridzinized dog, only about 70 per cent of i-alanin is so converted. The conclusion from this evidence is that certain monamino acids are more likely than others to escape into the urine when present in the blood in excessive quantities. What is the limit of concentration, beyond which the several amino-acids escape the kidney barrier, is not yet known.

Determined by reliable[1] methods, the absolute amount of monamino-acid nitrogen, in the urine of normal persons on a mixed diet, is from 0.15 to 0.30 gram per day, or 1.5 to 3 per cent of the total nitrogen. Henriques (26) gives 2.2 per cent as the average of a large number of determinations by the titration method. Levene and Van Slyke (27) give 1.1 to 2.8 per cent as the limits obtained by Van Slyke's gasometric method. Falk and Hessky (28) found from 1.9 to 2.8 per cent; and Landsberg (29) 2.5 to 3 per cent by the titration method, etc.

Both physiologically and pathologically, the amount may vary from this standard under special conditions. For example, in normal pregnancy (30), where the amount of total nitrogen excreted is reduced, owing to retention of that fraction which ordinarily is excreted as urea, the percentage of amino-acid nitrogen may rise very perceptibly. On a milk diet (31) also the fraction of undetermined nitrogen, which includes the amino-acid nitrogen, is higher than on ordinary mixed diet. (See table below, p. 68.)

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[1] The only methods which to-day can be regarded as reliable are the titration method of Sörensen, the gasometric method of Van Slyke, and, possibly, the colormetric method of Harding and MacLean (25).

Levene and Van Slyke (27) have examined urines from a considerable number of cases of cancer, arthritis, multiple sclerosis, gout, progressive muscular dystrophy, liver cirrhosis, nephritis, etc. The only conditions which showed abnormal percentages of amino-acid nitrogen were carcinoma, arthritis, gout, and nephritis; in other words, pathological states which involve either an abnormal breakdown of protoplasm or defective elimination. The authors state that "high results were usually due to albumin."

From the foregoing it would appear that the causes which might lead to an abnormally high excretion of monamino acids would be (1) impaired digestion, leading to absorption of products which could not be assimilated; (2) imperfect deamination; (3) excessive waste of body protein; (4) renal disturbances, accompanied by albuminuria.

2. *Hippuric acid.*—When benzoic acid is introduced into the blood stream by any channel whatsoever it combines in the kidney, and in some other tissues, with glycocoll to form hippuric acid. Benzoic acid is constantly being produced in small quantity in the human intestine when a mixed diet is consumed; consequently hippuric acid may under these circumstances always be demonstrated in the urine. Salts of benzoic acid are always present in many articles of human food, such as fruits and coarse vegetables—particularly in the skins and raw fiber—and in the intestine these salts are set free by digestion, and being absorbed, increase the production of hippuric acid. While, however, it constitutes but a small fraction of the nitrogenous bodies in human urine, hippuric acid forms a predominant part in the urine of herbivorous animals and is never absent from it even in starvation. The main source of the benzoic acid in such urine is certain phenol compounds found in the raw fiber of the coarse vegetable matter of the food. A part, however, may originate by putrefaction from proteins in the presence of carbohydrates. Accordingly benzoic acid may occasionally be found in the urine of carnivora. In man the conditions for an unusual production of hippuric acid then would be excessive putrefaction on a diet containing much raw fiber, or fruit and a plentiful amount of carbohydrate. According to Lewin (10) the administration of 100 grams of cane sugar, to convalescents on a milk and vegetable diet, greatly increased the excretion of hippuric acid.

Phenaceturic acid, which occurs occasionally in human urines, likewise depends upon intestinal putrefaction.

The amount of hippuric acid normally present in human urine varies from 0.1 to 2.0 grams (10). Since the nitrogen constitutes only 7.8 per cent of the entire molecule, it follows that hippuric-acid nitrogen is a very small fraction of the total nitrogen.

The most recent, as well as the most complete, study of the hippuric acid in human urine occurs in the report of the Remsen Board on the "influence of sodium benzoate on nutrition and health" (32). Since the substance there under investigation yields a direct precursor of hippuric acid, it was necessary to establish normal figures for the excretion of this body in healthy men. A few figures, quoted from the separate reports of Chittenden and Herter, will suffice. In the New Haven laboratory a certain subject, H. H. G., a man of 50 kilos weight, while on mixed diet yielding 11.34 grams total nitrogen, excreted an average of 0.064 gram hippuric-acid nitrogen per day, or 0.55 per cent of the total. J. V. L., a man of 67 kilos excreting 9.94 grams nitrogen, eliminated 0.046 gram of hippuric acid in 24 hours, or 0.48 per cent. The method employed was that originally devised by Schmiedeberg and Bunge (34), and modified later by Lewinski (35). In Herter's laboratory the same method was used and yielded results as high as 0.6 to 0.7 per cent of the total nitrogen.

H. B. Lewis (33) has recently published, for a normal subject who was consuming a diet of milk, cane sugar, and butter, a few results on the hippuric acid (determined by the method of Folin and Flanders (43), which agree very well with those just mentioned.

It may be stated, therefore, that an excretion of 1 per cent or more of the total nitrogen in the form of hippuric acid would be unusually high.

3. "*Peptid-bound*" *nitrogen.*—This designation is used by Henriques and Sörensen (36) for such nitrogen as can be unlinked from peptidlike combinations by hydrolysis of the urine with concentrated hydrochloric acid. Set free as monamino acids, this nitrogen could then be estimated by titration, by the formol method, or could be measured gasometrically. Some of the bodies yielding monamino acids in this way would be the oxyprotëic acids of Bondzynski (37) and his colleagues: diamino acids, such as histidin and other imidozol compounds; the peptidlike body described by Abderhalden and Pregl (38), which they isolated by dialysis from the alcoholic extract of urine and which yields on hydrolysis benzoic acid, alanin, phenylalanin and leucin.

The amount of these substances has been variously estimated. Bondzynski and Gottlieb (37) located from 2 to 3 per cent of the total nitrogen in their three oxyprotëic acids. Ginsburg (39) came to the conclusion that from 3 to 5 per cent of the total nitrogen may be present in this form even in health; but Solomon and Saxl (40), who traversed the same ground more carefully, limited the amount to 2.1 per cent of the total nitrogen in normal urines, and 2.5 to 3.5 per cent in carcinoma and late pregnancy. Henriques and Sörensen found only from 1 to 2 per cent of the total nitrogen in the entire so-called peptid-bound fraction of normal urines. It has been reported higher than this in the abnormalities of pregnancy, by Falk and Hessky and Landsberg. Definite limits for healthy individuals can not well be agreed upon until the methods are rendered somewhat more precise. In summary, it may be said that the normal limits of these fractions of the nitrogen are very narrow. All told, they might well constitute as low as 3 per cent of the total nitrogen, and would not be considered abnormally high if they yielded a total of 5 per cent. Above this, judging by existing data, suspicion might be aroused.

PLAN OF INVESTIGATION.

The plan of investigation contemplated, in the first instance, examination of the urines of patients exhibiting the disease in different degrees of severity, over a sufficient period of time to permit of some change for better or worse. Some of these patients were to be placed upon a low protein dietary, others on moderately high protein. Secondly, it was hoped to observe any effects on these fractions which the use of vitamines might have. Difficulties experienced by Dr. Voegtlin in the purification of these materials, however, prevented the accomplishment of more than a few scattered observations along this line. Finally, it was possible, through the cooperation of Mr. Givens, to observe two patients for a considerable period both on low and on high protein diets. The results on these two patients will be presented first.

In carrying out the first plan, urines were collected from about a dozen patients, once in two weeks, for a period of six to eight weeks: that is, three, and in a few cases four, 24-hour urines were taken at intervals of two weeks. During the whole of this time the patients were, with few exceptions, on a constant diet. From several of the same patients urines were again taken after the lapse of a month. Meantime several of them had shown very evident clinical improvement. (See below.) The conditions necessary for correlation of any marked change in the urine with the clinical status were therefore fulfilled.

The urines were collected under the strictest control possible. On collection days the patients were not permitted to leave the wards of the hospital; an attendant was placed in charge of the toilet for the male patients, and a nurse was especially delegated to keep watch of the collecting chambers in the female ward.

DIETS.

Two diets were in general use in the hospital when the writer joined the laboratory staff, on March 1, 1915. These were designated diet

A, or the corn-vegetable diet, and diet B, or the meat-egg-milk diet.[1] These diets had not been selected with any special reference to their influence on the urine, but rather with reference to their content of so-called vitamines or protecting substances. They served perfectly well, however, as low and moderately high protein diets. They were prescribed in the amounts of uncooked foods given below, the patient being requested to eat the entire amount served but being under no compulsion to do so. In every case the exact weight of cooked food served to each patient on individual dishes was determined and the amount remaining uneaten was weighed back immediately after the meal. By a proportion obtained from the known weights each day of the different articles before and after cooking in bulk the exact amount of each in terms of uncooked food was arrived at and is presented in Tables 1 and 2. This method seemed the most practical, considering the available number of nurses who could be delegated to the task of weighing the food on and off of the individual platters, although it is open to at least two sources of error; namely, the impossibility, with certain kinds of food, of having a homogeneous bulk of cooked food from which each serving would represent the average composition, and, second, the rather rapid loss in weight by evaporation during the time of eating, in the case of foods served hot. These sources of error, however, would tend to average out in comparing different patients; hence are not to be considered as very serious in work of the kind reported here.[2]

The following tables give the amounts of uncooked foods in the daily ration, the number of grams of protein, fat, and carbohydrate, and the amount of potential energy supplied by each article. The protein, with the single exception of turnip tops, is calculated from the analyses for total nitrogen made by Hunter, Givens, and Lewis in this laboratory (2). With the few exceptions noted in the tables, the figures for fat and carbohydrate are calculated from average analyses given by Atwater and Bryant (41).

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[1] A third emergency diet known as diet C, consisting of milk, cream, calf's liver, calf brains, and a few other ingredients in variable amount according to the ability of the patient to tolerate them, was employed at times. Only one patient of the present series was on this diet during the examinations here reported. For this reason no accurate description of this diet is necessary.

[2] Our main reliance was upon the conscientious care with which the original weighings of uncooked and cooked foods were done by the head nurse, Miss Maude Fauquier, and the good judgment shown by her in distributing the food from pot to individual dishes.

TABLE 1.—*Diet A, corn-vegetable diet.*

Weight of uncooked food.	Article.	Protein.	Fat.	Carbohydrate.	Calories.
Grams.		*Grams.*	*Grams.*	*Grams.*	
300	White bread	24.9	4.0	157	783.0
30	Butter	.49	21.0		225.2
100	Cabbage (raw), served stewed with pork fat	1.15	.3	5.5	30.0
50	Corn meal, served fried in pork fat	5.30	.9	36.8	181.0
25	Ham, boiled, lean	6.43	9.0		110.1
75	Hominy grits, served boiled	6.13	1.0	[1] 59.3	277.6
30	Corn syrup			[2] 23.4	95.9
50	Pork fat, residue after cooking	.9	41.0		385.0
150	Potatoes, Irish or sweet	2.5		25.0	112.7
30	Prunes, dried, seeded, served stewed	.4		22.0	91.8
100	Turnip tops, served boiled	4.2	.6	6.3	48.6
40	Cane sugar			40.0	164.0
40	Whole milk	1.4	1.6	2.0	28.8
350	Coffee				
	Total	53.8	82.4	377.3	2,533.7

[1] Determined by R. H. Harries in this laboratory.
[2] From Gephart and Du Bois, Arch Int. Med., 1915, May, p. 829.

TABLE 2.—*Diet B, meat-egg-milk diet.*

Weight of uncooked food.	Article.	Protein.	Fat.	Carbohydrate.	Calories.
Grams.		*Grams.*	*Grams.*	*Grams.*	
300	White bread	24.9	4.0	157.0	783.0
45	Butter	.7	36.0		337.7
50	Corn meal, served as mush	5.3	.9	36.8	181.0
100	Eggs, yolk and white beaten	13.4	10.0		147.9
100	Lean beef, steak, or oxheart	20.0	8.0		156.4
100	Orange juice			[1] 7.8	32.0
150	Potatoes, Irish and sweet	2.5		25.0	112.7
30	Prunes, dried, seeded, served stewed	.4		22.0	91.8
40	Cane sugar			40.0	164.0
1,000	Whole milk	34.0	38.0	50.0	697.8
	Total	101.2	96.9	338.6	2,704.3

[1] Determined by R. H. Harries in this laboratory.

Brief description of cases.

Case No. 1 (*A. L.*).—Admitted August 17, 1914. White, female, age 36, married. Mild case. Probably first attack. Slight erythema on neck, dorsum of both hands glazed. Memory slightly defective.

Case No. 2 (*G. O. C.*).—Admitted August 17, 1914. White, male, age 22, single. Recurrence, moderate. Erythema on fingers about joints, cuff at wrist, slight about elbows. Erythematous papillae and comedones on forehead. Erythema on neck. No mental symptoms. Discharged February 14, 1915. Readmitted March 3, 1915.

Case No. 14 (*W. A. McD.*).—Admitted August 15, 1914. White, male, age 58, married. Severe case. Desquamation over elbow. Erythema over both hands. Erythema on side of neck, posterior to ears, skin rough and desquamating. Skin over face and forehead rough. Mental symptoms at one time.

Case No. 36 (*W. J. McK.*).—Admitted November 2, 1914. White, male, age 55, married. Severe case. Mental symptoms. Skin of right hand and wrist rough and red. Palmar surface of fingers and hands scaly. Mucosa of mouth anaemic. Feet burn and sting at times.

Case No. 48 (M. S.).—Admitted February 2, 1915. White, female, age 33, married. Three children living. Recurrence, mild case with exaggerated nervous symptoms. Dermatitis over back of hands, wrists, and face. Darkening and dermatitis over knees. Mucosa of lower lip inflamed. Mental symptoms.

Case No. 59 (E. S.).—Admitted February 26, 1915. White, female, age 39, married. Four children living, one dead. Mild case; first attack. Dermatitis on back of hands, especially on knuckles; also wrists and elbows. No mental symptoms. Discharged improved.

Case No. 60 (Miss E.).—Admitted March 3, 1915. White, female, age 22, single. First attack: Mild. Desquamation over hands, knuckles, some erythema on elbows. Tongue appears normal. No mental symptoms.

Case No. 69 (L. B.).—Admitted March 29. White, female, age 24. Some weakness of knees. Vertigo when walking, some anæmia. Tongue slightly reddened and denuded at margin. Erythema over knuckles and to first joint of fingers. Erythema over chin. Mental depression.

Case No. 70 (S. E. T.).—Admitted April 1, 1915. White, male, age 49, married. Recurrence, moderate. Erythema about upper end of sternum, beneath each eye and on back of hands. No mental symptoms.

Case No. 77 (J. J. F.).—Admitted February 22, 1915. White, male, age 51. First attack, mild. Dermatitis on back of hands. No mental symptoms.

Case No. 51 (M. R.).—Admitted February 11, 1915. White, female, age 30, married. Five children living, one dead. First attack, mild. Erythema over the dorsum of hands and forearms, and slight erythema on neck. No mental symptoms.

Case No. 52 (C. C. S.).—Admitted February 15, 1915. White, male, age 34, married. Six children living. Recurrence moderate. Erythema and desquamation over back of hands and wrist, redness of elbows; tongue very much reddened. No mental symptoms.

Case No. 57 (V. I. C.).—Admitted February 26, 1915. White, male, age 47, married. Five children living, one dead. Recurrence, moderate. Erythema on back of both hands. Pigmentation about elbows. No mental symptoms.

Case No. 66 (F. S. C.).—Admitted March 21, 1915. White, male, age 30, married. Three children living. Recurrence, mild. Erythema on back of hands, slight desquamation over knees. No mental symptoms. Discharged improved.

Case No. 73 (J. G. H.).—Admitted April 7, 1915. White, male, age 36, single, severe case; first attack. Erythema on dorsal surface of the hands, thumbs, and knuckles, and flexor surface of the wrist and elbows. Slight mental symptoms.

Case No. 81 (E. W. M.).—Admitted April 20, 1915. White, male, age 48, married. Three children living, one dead. Mild case; recurrence. Back of hands slightly pigmented and glazed.

Case No. 82 (A. J. C.).—Admitted April 20, 1915. White, male, age 50, married. Six children living. Recurrence moderate. Marked erythema back of both hands and wrist. Mental confusion, elation.

Case No. 83 (J. A. J.).—White, male, age 68. Widowed. Admitted May 4, 1915. Tongue slightly fissured, margins slightly redder than normal. Constipated. Skin: Some erythema on dorsum both hands. Dry, slight desquamation, glazed. No pellagra skin lesion elsewhere. Mental condition good.

June 12 to 30. Patient at no time confined to bed except on June 29 and 30. General condition showed slow improvement. Tendency to constipation. After substitution of cheese for meat in diet, patient complained of obstinate constipation, partially relieved by soapsuds enemata. Pellagra condition improved on discharge.

METHODS OF ANALYSIS.

The total acidity in the urine was determined by the well-known method of Folin; the indican by the colorimetric method adopted in this laboratory by Hunter, Givens, and Lewis (2); the total nitrogen by Kjeldahl's method, using only copper sulphate with concentrated sulphuric acid in hydrolysis; monamino acids by the formol titration of Henriques and Sörensen, employing the modification of Benedict and Murlin (42). This fraction of the nitrogen is designated in the table as "formol-titration" nitrogen. The hippuric acid was determined by the method of Folin and Flanders (43). Polypeptid nitrogen, or following the designation of Henriques and Sörensen (44), the peptid-bound nitrogen was found by titration with formalin, following digestion of the urine, reduced to small volume for at least three hours with concentrated hydrochloric acid. This method, as originally described, proceeds upon the assumption that all the hippuric acid can be removed by shaking the acidified urine six times with ethyl acetate. This having been done, the urine is reduced to small volume, is digested with 50 c. c. concentrated HCl over wire gauze for three hours, is dried down, taken up in sodium hydroxide, the ammonia separately determined in one fraction while the other is prepare for titration with formalin, for free ammonia and amino-acid groupings. Folin and Flanders (43) have drawn attention to some of the inaccuracies of this method of removing hippuric acid, however, and in the present work (see tables below) it has been found that if the total hippuric-acid nitrogen and the free amino-acid nitrogen, which can be estimated by titration after treatment with formalin, are subtracted from the total-titration nitrogen, after digestion with 50 c. c. HCl and removal of ammonia, in many urines nothing remains. This means either that Henriques and Sörensen, and those who have followed their technique, have failed to remove all of the hippuric acid, or that the method of Folin and Flanders finds quite too much hippuric acid. That the former is probably the correct interpretation, is seen from the fact that more vigorous digestion with 80 c. c. concentrated HCl discloses a larger amount of titratable nitrogen. In the tables which follow it will be noted that very little peptid-bound nitrogen was found in any of the urines in the month of March and April, but that in several of the June urines the percentage amounts correspond fairly well with those reported by Henriques and Sörensen (p. 50). The reason is that the June urines were subjected to more rigorous digestion. The interpretation just given is borne out also by the control determination of benzoic acid added to urine, to be mentioned presently, and by comparison of the amount of hippuric acid obtained by Dakin's method (44).

The final method of digestion for peptid-bound nitrogen has actually been carried out a little differently from that recommended by Henriques and Sorensen, and it is possible that this modification is responsible to some degree for the larger yield. From 100 to 200 c. c. (according to dilution) of the day's urine were placed in a 500 c. c. Kjeldahl flask and, after adding 25 c. c. concentrated HCl, were boiled down to about 25 c. c. total volume. An additional amount of acid, 25 to 50 c. c., was then added, Hopkins condensers were introduced, and the digestion carried on, over wire gauze and with just sufficient flame to keep up gentle boiling, for three to three and one-half hours.

Since by Benedict and Murlin's modification of the formoltitration method ammonia is removed by precipitation with Merck's phosphotungstic acid, it is important to make sure that all ammonia is thus removed after digestion before the final titration. This has been done with a great many of the urines by aeration of 20 c. c. of the final filtrate, by Folin's ammonia method, for four hours. The water pressure in the laboratory was not all that could be desired during the course of this work, and yet the fact that the amount of ammonia found in this filtrate was never more than a few milligrams (calculated for the entire day's urine) shows that the aeration was sufficient for the purpose. Also it was found sufficient to remove the ammonia from a N/10 NH_4Cl solution in three to four hours.

Controls of methods employed.

1. *Monamino acid method.*—So far as the present writer's experience goes, only Merck's phosphotungstic acid[1] can be relied upon to remove all the ammonia from a urine. The usual test of this quality and proof that no glycocoll is removed by the precipitation is to add an assayed sample of glycocoll to the urine and recover it in the final titration. The following test shows that the reagents to be used met these requirements:

(*a*) 100 c. c. urine+20 c. c. N/10 glycocoll+80 c. c. phosphotungstic solution.
(*b*) 100 c. c. urine+20 c. c. H_2O+80 c. c. phosphotungstic acid solution.

Number cubic centimeters N/10 NaOH required on final titration for 50 c. c. filtrate:

(*a*)	14.60	
	14.60	
Average		14.60
(*b*)	10.50	
	10.50	
Average		10.50
Difference		4.10
Theory		4.15

2. *Peptid-bound nitrogen.*—As already stated, the method of digestion of the urine adopted by Henriques and Sörensen yields variable results. On this account, one could not hope for more than to show whether an unusual amount of polypeptid nitrogen may occur in the urines of pellagrous subjects. The following test indicates the accuracy which may be expected:

(*b*) 100 c. c. urine+20 c. c. N/10 glycocoll.
(*a*) 100 c. c. urine without glycocoll.
Both treated as stated on page 55.

Final titration (c. c.):

(*b*)	10.9	
	11.1	
Average		11.0
(*a*)	7.9	
	8.0	
Average		8.0
Difference		3.0
Theory		4.15

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[1] Through the courtesy of Prof. Graham Lusk a sufficient quantity of this product was placed at my disposal by the department of physiology of Cornell Medical College in New York City.

Only 75 per cent of glycocoll is recovered.

It is probable that glycocoll was lost by conversion to ammonia and precipitation with the stronger phosphotungstic acid solution required by the large amount of ammonium salts after digestion.

3. *Recovery of benzoic acid.*—The only difficult step in the Folin-Flanders method for estimation of hippuric acid lies in the exact determination of the end point. The following may be given as an example of the accuracy attainable with this method:

(*a*) 200 c. c. urine.
(*b*) 200 c. c. urine+100 c. c. N/140 benzoic acid solution.
Treated exactly alike according to direction of Folin and Flanders.

Titration of solution N/10 sodium alcoholate (c. c.):

(*b*)	34. 6
(*a*)	16. 2
Difference	18. 4
Theory (error 5 per cent plus)	19. 44

The end point with toluol as solvent, the writer has since found, is somewhat sharper than this. All determinations here reported are subject to an error of the order just noted. The chloroform used was always freshly washed, and other possible errors were eliminated by blank determinations of acidity on freshly washed chloroform carried through the ammonium sulphate and saturated sodium chloride solutions.

DISCUSSION OF RESULTS.

It will be most convenient to present first the effects on the urine of changing the same patient from the corn-vegetable diet (A) to a milk-egg-cheese diet (modified diet B). Two patients (No. 81 and No. 83), selected because the disease was rather quiescent at the time, permitting therefore a sufficiently rigorous adherence to the same regimen of food, were thus examined. Cheese, rather than meat, for the high protein period was selected for the purpose of following the endogenous purin metabolism. Mr. Givens has made these observations; and the writer is under obligation to him for permission to use his figures for the total nitrogen and for the gastric analyses given in the footnotes to the tables.

TABLE 3.—*Case No. 81 (E. W. M.).*

Date.	Wheat bread (gms.).	Butter (gms.).	Boiled cabbage (gms.).	Eggs (gms.).	Corn-meal mush (gms.).	American cheese (gms.).	Boiled hominy (grits) (gms.).	Karo (gms.).	Orange juice (c. c.).	Pork fat (gms.).	Boiled potatoes (gms.).	Stewed prunes (gms.).	Stewed turnip tops (gms.).	Cane sugar (c. c.).	Whole milk (c. c.).	Coffee (c. c.).
DIET A.																
May 4–5	300	15	108	.....	110	15	440	30	.....	16	150	52	....	40	40	....
5–6	300	30	111	.....	104	15	380	30	.....	17	150	56	79	40	40	....
6–7	300	30	108	.....	98	15	373	30	.....	17	150	56	....	40	40	....
7–8	300	30	110	.....	104	15	366	30	.....	17	150	43	....	40	40	....
8–9[1]	300	30	104	.....	104	15	357	30	.....	15	150	43	88	40	40	....
9–10[1]	300	30	104	.....	111	15	388	30	.....	17	150	43	84	40	40	....
10–11[1]	300	30	105	.....	98	15	390	30	.....	18	150	43	68	40	40	....
11–12	300	30	94	.....	115	12	387	30	.....	15	150	56	76	40	40	....
DIET B WITHOUT COFFEE.																
May 12–13	300	45	.....	98	221	80	.....	....	100	....	150	56	....	40	1,000	....
13–14	300	45	.....	98	248	80	.....	....	100	....	150	56	....	20	1,000	....
14–15[1]	300	45	.....	98	223	80	.....	....	.....	....	150	56	....	40	1,000	....
15–16[1]	200	30	.....	98	245	80	.....	....	100	....	150	55	....	40	1,000	....
16–17[1]	200	30	.....	100	251	80	.....	....	100	....	150	55	....	40	1,000	....
17–18[1]	300	45	.....	98	226	80	.....	....	100	....	150	55	....	40	1,000	....
18–19[1]	300	45	.....	100	255	80	.....	....	100	....	150	55	....	40	1,000	....
19–20	300	45	.....	98	234	80	.....	....	100	....	150	45	....	40	1,000	....
DIET B WITH COFFEE.																
May 20–21	300	45	.....	100	288	80	.....	....	100	....	150	45	....	40	1,000	350
21–22	300	45	.....	100	277	80	.....	....	100	....	150	45	....	40	1,000	350
22–23[1]	300	45	.....	98	275	80	.....	....	100	....	150	45	....	40	1,000	350
23–24[1]	300	45	.....	100	248	80	.....	....	100	....	150	49	....	40	1,000	350

[1] Urines analyzed only on these days.

TABLE 4.—*Analysis of urines, case No. 81 (E. W. M.).*[1]

Date.	Volume.	Specific gravity.	Reaction.	Total nitrogen.	Formol titration nitrogen.	Per cent of total nitrogen.	Hippuric acid nitrogen.	Per cent of total nitrogen.	Peptid bound nitrogen.	Per cent of total nitrogen.
	c. c.									
May 8–9	700	1.022	Acid	7.42	0.090	1.2	0.142	1.92	0.140	1.89
9–10	995	1.017	do	7.52	.078	1.03	.140	1.86	....	....
10–11	1.020	1.018	do	7.44	.074	1.0	.126	1.69	....	....
Average	....	....	....	7.46	.081	1.1	.136	1.8	....	....
14–15	1,025	1.022	Acid	11.81	.093	.79	.118	1.0	.137	1.16
15–16	1,005	1.021	do	12.31	.140	1.14	.170	1.38	....	....
16–17	990	1.024	do	13.49	.167	1.24	.130	.96	....	....
17–18	940	1.026	do	14.25	.170	1.19	.123	.86	....	....
18–19	958	1.027	do	14.28	.256	1.8	.185	1.29	....	....
Average	....	....	....	13.22	.165	1.24	.145	1.1	....	....
21–22	1,040	1.025	Acid	13.07	.205	1.53	.209	1.56	....	....
22–23	805	1.026	do	11.38	.174	1.52	.187	1.64	....	....
23–24	900	1.026	do	11.68	.176	1.50	.207	1.72	....	....
Average	....	....	....	12.04	.185	1.52	.201	1.66	....	....

[1] Gastric analysis on May 26 showed total acidity 20, free acidity 0, pepsin 0.

TABLE 5.—*Daily food, case No. 83 (J. A. J.).*

Date.	Wheat bread.	Butter.	Cabbage.	Corn-meal mush.	Eggs.	Hominy.	Karo.	Peaches.	Pork fat.	Potatoes.	Prunes.	Turnip tops.	Sugar.	Milk.	Coffee.	American cheese.
DIET A.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Cc.*	*Cc.*	*Cc.*	*Gm.*
June 11	300	30	96	50	.....	72	30	.....	45	150	30	100	40	40	.....	30
13	300	30	100	50	.....	75	30	.....	46	150	30	100	40	40	.....	30
14	300	30	98	75	.....	93	30	.....	45	150	23	99	40	40	.....	30
15	300	30	106	50	.....	40	30	.....	47	150	30	74	40	40	.....	30
16	300	30	107	50	.....	75	30	.....	47	150	30	100	40	40	.....	30
17[1]	300	30	100	52	.....	75	30	.....	45	150	30	99	40	40	.....	30
18[1]	300	30	99	50	.....	80	30	.....	46	150	30	100	40	40	.....	30
19	300	30	100	78	.....	70	30	.....	47	150	30	93	40	40	.....	30
MODIFIED DIET B.																
June 20[1]	300	45	.....	58	100	....	....	100	....	150	30	.....	40	840	.....	80
21[1]	300	45	.....	50	100	....	....	100	....	150	30	.....	40	1,000	.....	80
22	300	45	.....	50	100	....	....	.....	....	150	30	.....	40	1,000	.....	80
23[1]	300	45	.....	50	100	....	....	.....	....	150	30	.....	40	1,000	.....	80
24	300	45	.....	50	100	....	....	100	....	150	30	.....	40	1,000	.....	80
25[1]	300	45	.....	54	100	....	....	100	....	150	38	.....	40	1,000	.....	80
26	300	45	.....	48	100	....	....	100	....	150	30	.....	40	1,000	.....	80
27[1]	300	45	.....	48	100	....	....	100	....	150	30	.....	40	1,000	.....	80
MODIFIED DIET B, WITH COFFEE.																
June 28	300	45	.....	43	100	....	....	100	....	150	30	.....	40	1,000	350	80
29[1]	280	45	.....	50	100	....	....	100	....	150	30	.....	40	1,000	350	80

[1] Urines analyzed only on these days.

TABLE 6.—*Analysis of urines, case No. 83 (J. A. J.).*[1]

Date (1915).	Volume.	Specific gravity.	Reaction.	Total nitrogen.	Formol titration nitrogen.	Per cent of total.	Hippuric acid nitrogen.	Per cent of total.	Peptid bound nitrogen.
	c. c.								
June 16–17	1,540	1,015	Acid	6.092	0.102	1.7	0.148	2.4	....
17–18	1,660	1,013	...do	6.48	.082	1.2	.127	2.0	....
19–20	1,020	1,019	...do	5.506	.080	1.5	.135	2.4	....
Average	....	....	....	6.025	.088	1.5	.136	2.3	....
DIET B, MODIFIED WITHOUT COFFEE.									
June 20–21	1,220	1,020	Acid	7.998	.083	1.0	.160	2.0	....
22–23	1,275	1,020	...do	9.160	.076	.8	.132	1.4	....
24–25	1,470	1,020	...do	11.660	.106	.9	.159	1.3	....
Average	....	....	....	9.606	.088	.9	.150	1.6	......
DIET B, MODIFIED WITH COFFEE.									
June 26–27	1,890	1,016	Acid	13.120	.084	.6	.192	1.5	......
28–29	1,870	1,017	...do	14.360	.112	.8	.168	1.2	......
Average	....	....	....	13.74	.098	.7	.180	1.4	......

[1] Gastric analysis on June 11 showed total acidity 86, free acidity 59, pepsin 20. According to Mr. Givens's results on normal subjects, only the pepsin in this analysis would be considered low.

These two cases present the contrast of low acidity and pepsin number in the stomach contents on the one hand (No. 81), and a normal gastric juice (No. 83) on the other. It is possible that this circumstance offers an explanation of the chief difference in the reaction of the two to the change of diet, namely, a much higher elimination of monamino nitrogen in the case of No. 81 on the high protein diet. With an average of 7.46 grams total nitrogen in the urine, No. 81 eliminated 0.081 gram formol-titration nitrogen, while with 13.22 grams average total nitrogen he eliminated 0.165 gram formol-titration nitrogen. No. 83 on the low protein diet yielding an average of 6.02 grams nitrogen in the urine eliminated 0.088 gram formol-titration nitrogen, but when the total nitrogen rose to 9.6 grams the amino-acid nitrogen had not yet risen at all (average) and when the average total was 13.7 grams the amino-acid nitrogen was only 0.098 gram.

The polypeptid nitrogen shows a similar contrast. Owing to an accident, in which most of the urine bottles of No. 81 were broken before this fraction of the urine was completed, the writer is able to present figures for only two days. These two days, however, are sufficient to warrant the statement that a considerable amount of peptid-bound nitrogen was present in the urine even before the high protein diet was started. In the urines of No. 83, on the other hand, not a trace of peptid-bound nitrogen could be detected at any time, in spite of the most vigorous hydrolysis with HCl.

In Table 11 will be found further facts bearing on this relationship of gastric condition to the peptid-bound nitrogen in the urine.

As regards the hippuric acid, both patients responded in the same way. The change to a high protein diet increased the absolute output, but not as much as the total nitrogen. This result would be expected if the higher protein supplied not a proportionate amount of benzoates or other precursors of hippuric acid but merely a more favorable medium in the gut for putrefactive processes which might form the cleavage of various precursors. (See p. 49.)

A second marked increase is seen in both cases following the ingestion of coffee. Lewin quotes Meissner and Shephard (46) as stating that kinic acid ($C_7H_{12}O_6$), a derivative of hexamethylene found in the coffee bean, increases the output of hippuric acid in the urine.

THE AMINO FRACTIONS OF THE URINE IN RELATION TO THE CLINICAL CONDITION OF THE PATIENT.

In Tables 7 and 8 are presented full details of the dietaries consumed by these patients for two days immediately preceding collection of the urine, the day of collection, and the day following. In Tables 9 and 10 will be found the detailed analyses of the urines. Along with these details are given full clinical notes, supplied by Dr. Grimm, as to the condition of the patients at or near the time of collection of the urine.

TABLE 7.—*Daily amounts of food actually eaten by patients on diet A.*

[Figures are given for one or two days previous to urine day, the urine day, and the day following.]

Date.	Net body weight.	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy grits.	Corn sirup.	Fat pork.	White or sweet potatoes.	Prunes.	Turnip tops.	Cane sugar.	Milk.	Coffee.	Concentrated extract autolyzed yeast.
Case No. 1:																
1915.	*Kilos.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*C.c.*	*C.c.*	*C.c.*
Mar. 22	......	145	30	100	50	25	10	30	36	150	30	.....	40	40	350	.....
23	......	166	30	100	50	25	12	30	36	150	30	.....	40	40	350	.....
24 [1]	62.8	193	30	78	50	25	16	30	35	150	30	.....	40	40	350	.....
Apr. 5	......	280	30	105	50	25	14	30	40	150	30	.....	40	40	350	.....
6	......	265	30	95	50	25	10	30	39	30	30	95	40	40	350	.....
7 [1]	62.6	231	30	99	50	25	15	30	37	150	30	.....	40	40	350	.....
18	......	275	30	109	42	25	11	30	30	150	30	.....	40	40	350	.....
19	......	284	30	99	50	25	9	30	41	150	30	14	40	40	350	.....
20 [1]	62.9	229	30	56	50	25	13	30	35	150	30	99	40	40	350	.....
June 4	......	300	30	100	50	25	14	30	47	150	30	100	40	40	350	.....
5	......	300	30	100	50	25	9	30	33	150	30	100	40	40	350	.....
6	......	300	30	90	19	25	31	30	26	123	30	.....	40	40	350	.....
7 [1]	62.5	300	30	100	50	25	16	30	47	150	30	100	40	40	350	.....
Case No. 51:																
Mar. 20	......	285	30	100	50	25	45	30	41	150	30	100	40	40	350	.....
23	......	275	30	100	50	25	26	30	41	150	30	100	40	40	350	.....
24 [1]	52.5	226	30	100	50	25	45	30	43	150	30	100	40	40	350	.....
Apr. 4	......	300	30	100	50	25	73	30	45	150	30	100	40	40	350	.....
5	......	300	30	105	50	25	75	30	45	150	30	105	40	40	350	.....
6 [1]	52.5	271	30	104	50	25	74	30	47	150	30	95	40	40	350	.....
Case No. 51:																
Apr. 19	......	300	30	99	50	25	75	30	49	150	30	100	40	40	350	.....
20	......	300	30	99	50	25	75	30	49	150	30	99	40	40	350	.....
21 [1]	54.7	256	30	99	50	25	69	30	43	72	30	90	40	40	350	.....
June 4	......	300	30	99	50	25	74	30	47	150	30	100	40	40	350	.....
5	......	300	30	100	50	25	75	30	41	150	30	64	40	40	350	.....
6	......	300	30	90	19	25	77	30	38	150	30	99	40	40	350	.....
7 [1]	56.1	276	30	100	50	25	75	17	49	150	30	100	40	40	350	.....
Case No. 52:																
Mar. 21	......	300	30	100	50	25	75	30	41	150	30	100	40	40	350	.....
22	......	300	30	100	50	25	75	30	41	150	30	100	40	40	350	.....
23	......	300	30	100	50	25	75	30	41	150	30	100	40	40	350	.....
24 [1]	49.6	248	30	100	50	25	75	30	38	122	20	.....	40	40	350	.....
Apr. 3	......	300	30	104	50	25	74	30	28	150	30	107	40	40	350	.....
4	......	300	30	100	50	25	73	30	35	150	30	11	40	40	350	.....
5	......	300	30	105	50	25	73	30	45	150	30	105	40	40	350	.....
6 [1]	49.7	300	30	95	50	25	74	30	40	150	30	62	40	40	350	.....
17	......	300	30	94	50	25	65	30	46	150	30	99	40	40	350	35
18	......	300	30	98	50	25	71	30	38	150	30	95	40	40	350	35
19	......	300	30	99	50	25	75	30	47	150	30	100	40	40	350	35
20 [1]	50.1	285	30	99	50	25	75	30	46	57	30	99	40	40	350	35
Case No. 57:																
Mar. 21	......	300	30	.....	50	25	75	30	37	150	30	100	.....	40	300	.....
22	......	300	30	.....	50	25	75	30	25	150	30	100	40	40	350	.....
23	......	300	30	.....	50	25	75	30	21	150	30	100	.....	40	350	.....
24 [1]	55.0	253	30	.....	50	25	75	30	19	150	30	100	.....	40	300	.....
Apr. 3	......	300	30	104	50	25	74	30	41	150	30	105	.....	40	300	20
4	......	300	30	100	50	25	73	30	45	150	30	101	.....	40	325	20
5	......	300	30	105	50	25	76	30	46	150	30	105	.....	40	300	20
6 [1]	55.2	265	30	.....	50	25	74	30	22	150	30	95	.....	40	300	20
17	......	300	30	94	50	25	71	30	46	150	30	99	40	40	350	20
18	......	300	30	109	50	25	75	30	38	150	30	95	40	40	350	20
19	......	300	30	99	50	25	75	30	47	150	30	100	40	40	350	20
20 [1]	56.7	271	30	99	50	25	75	30	47	28	30	99	40	40	350	20
Case No. 60:																
Mar. 7	......	258	15	100	50	25	26	30	41	150	30	100	.....	.....	150	.....
8	......	300	30	100	50	25	45	30	42	150	30	100	.....	.....	150	.....
9	......	267	15	100	50	25	50	29	42	150	30	100	.....	.....	300	.....
10 [1]	52.5	280	30	100	50	25	56	30	34	150	30	16	20	20	150	.....
21	......	200	21	100	50	25	75	30	36	150	30	.....	.....	.....	300	.....
22	......	300	30	100	50	25	75	30	36	150	30	.....	.....	.....	300	.....
23	......	297	15	100	50	25	75	30	41	150	30	100	20	20	325	.....
24 [1]	56.4	284	15	100	50	25	75	30	26	150	30	.....	20	20	325	.....
Apr. 4	......	298	15	100	50	25	73	30	42	150	30	101	40	40	175	.....
5	......	300	15	105	50	25	66	30	40	150	30	.....	40	40	350	.....
6	......	300	15	140	50	25	75	30	43	150	30	96	40	40	350	.....
7 [1]	58.7	300	30	.....	50	25	65	30	21	150	30	.....	40	40	350	.....

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[1] Day urine was delivered.

TABLE 7.—*Daily amounts of food actually eaten by patients on diet A*—Contd.

Date.	Net body weight.	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy grits.	Corn sirup.	Fat pork.	White or sweet potatoes.	Prunes.	Turnip tops.	Cane sugar.	Milk.	Coffee.	Concentrated extract autolyzed yeast.
Case No. 66:	*Kilos.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*C.c.*	*C.c.*	*C.c.*
Mar. 23	......	290	30	100	24	25	75	30	31	150	30	100	.....	.....	.....	.....
24	......	290	30	100	50	25	68	30	43	150	30	100	.....	.....	.....	.....
25	......	300	45	100	50	25	75	30	45	150	30	100	.....	.....	.....	.....
26[1]	64.0	275	30	100	50	25	75	30	43	150	30	100	20	20	175	.....
Apr. 3	......	300	30	104	50	25	69	30	40	150	30	104	20	20	175	.....
4	......	300	30	100	50	25	73	30	45	150	30	101	20	20	175	.....
5	......	300	30	105	50	25	72	30	46	150	30	105	40	40	350	.....
6[1]	64.1	300	30	95	50	25	44	30	48	150	30	95	20	20	175	.....
17	......	300	30	94	50	25	63	30	47	150	30	99	40	40	350	.....
19	......	300	30	101	49	25	75	30	48	150	30	100	40	40	350	.....
20[1]	63.7	260	30	54	50	25	75	30	44	150	30	70	40	40	350	.....
Case No. 69:																
Apr. 5	......	300	30	105	48	25	61	6	45	150	30	105	.....	40	300	.....
6	......	300	30	95	44	25	31	2	42	150	30	50	.....	40	300	.....
7	......	300	30	.....	50	25	66	14	42	57	30	99	.....	40	300	.....
8[1]	51.1	250	25	.....	49	25	20	.....	40	150	30	108	.....	40	300	.....
Case No. 70:																
Apr. 4	......	300	30	100	50	25	73	30	45	150	30	101	40	40	350	.....
5	......	300	30	105	39	25	89	30	40	150	30	105	40	40	350	.....
6	......	300	30	95	50	25	74	30	47	72	30	95	40	40	350	.....
7[1]	55.8	262	30	61	50	25	68	30	38	150	30	96	40	40	350	.....
18	......	300	30	109	50	25	75	30	38	150	30	95	40	40	350	.....
19	......	300	30	99	50	25	75	30	47	150	30	100	40	40	350	.....
20	......	247	30	.....	35	25	72	30	33	71	30	99	40	40	350	.....
21[1]	56.5	260	30	49	50	25	69	30	41	142	30	66	40	40	350	.....
Case No. 82:																
Apr. 21	......	300	30	104	50	25	46	30	39	150	30	16	40	40	350	50
22	......	297	30	100	50	25	70	30	45	150	30	100	40	40	350	50
23[1]	54.5	300	30	100	50	25	75	30	46	150	27	101	40	40	350	50
June 4	......	300	30	100	50	25	75	30	47	150	30	100	40	40	350	20
5	......	300	30	100	50	25	75	30	47	150	30	100	40	40	350	20
6	......	300	30	100	12	25	75	30	35	150	32	90	40	40	350	20
7[1]	55.4	360	54	100	60	25	90	30	60	180	36	222	40	40	350	20

[1] Day urine was delivered.

TABLE 8.—*Daily amount of foods actually eaten by patients on diet B.*

[Figures are given only for 2 days previous to urine day, the urine day, and the day following.]

Date.	Net body weight.	Wheat bread.	Butter.	Cornmeal.	Eggs.	Meat.	Orange juice.	Potatoes.	Prunes	Cane sugar.	Whole milk.	Coffee.
1915.												
Case No. 2:	*Kilos.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*C. c.*	*C. c.*
Mar. 8	......	300	45	50	100	100	100	150	30	40	1,000	350
9	......	300	45	50	100	100	100	150	30	40	1,000	350
10	......	300	45	50	100	100	100	150	30	40	1,000	350
11[1]	69.5	300	45	50	.....	100	100	150	30	40	840	350
22	......	300	45	50	100	100	100	150	30	40	1,000	350
23	......	300	45	50	100	100	100	150	30	40	1,000	350
24	......	300	45	50	100	100	100	150	30	40	1,000	350
25[1]	68.9	300	45	50	100	100	100	150	30	40	840	350
Case No. 14:												
Mar. 22	......	168	45	......	98	78	100	76	30	40	1,000	350
23	......	183	35	......	100	100	100	150	30	40	1,000	350
24	......	224	45	......	100	100	100	150	30	40	1,000	350
25[1]	56.8	275	36	......	100	100	100	......	30	40	1,000	350
Apr. 5	......	197	45	......	99	100	100	150	30	40	840	350
6	......	300	45	......	100	100	100	150	30	40	1,000	350
7	......	262	45	......	100	100	100	150	30	40	1,000	350
8[1]	57.4	150	30	......	100	13	100	21	30	40	1,000	350

[1] Day urine was delivered.

TABLE 8.—*Daily amount of foods actually eaten by patients on diet B*—Contd.

Date.	Net body weight.	Wheat bread.	Butter.	Cornmeal.	Eggs.	Meat.	Orange juice.	Potatoes.	Prunes.	Cane sugar.	Whole milk.	Coffee.
1915. Case No. 14—Contd.	*Kilos.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*C. c.*	*C. c.*
June 5	......	241	54	......	120	120	100	180	36	40	1,200	350
6	......	230	54	......	120	120	100	180	30	40	1,040	250
7	......	250	54	......	120	120	100	180	36	40	1,200	250
8 [1]	61.3	248	54	......	120	120	100	180	36	40	1,200	250
Case No. 36:												
Mar. 22	......	300	45	50	100	100	100	150	30	40	1,000	350
23	......	300	45	50	100	100	100	150	30	40	1,000	350
24	......	300	45	50	100	100	100	150	30	20	1,000	350
25 [1]	65.5	300	45	50	100	100	100	150	30	40	1,000	350
Apr. 5	......	300	45	50	100	100	100	150	30	40	840	350
6	......	300	45	49	100	100	100	150	20	40	800	350
7	......	300	45	50	100	100	100	150	30	40	1,000	350
8 [1]	65.7	250	45	50	100	100	100	150	30	40	1,000	350
June 5	......	360	54	......	120	170	100	180	36	40	1,200	350
6	......	360	54	60	120	120	100	180	30	40	1,200	350
7	......	360	54	60	120	120	100	180	36	20	1,200	175
8 [1]	69.5	360	54	60	120	120	100	180	36	40	1,200	350
Case No. 48:												
Mar. 22	......	300	45	50	100	100	100	150	30	20	1,000	175
23	......	300	45	50	100	100	100	150	30	20	1,000	175
24	......	300	45	50	100	63	100	150	30	20	1,000	175
25 [1]	49.9	292	31	50	100	......	100	11	30	20	1,000	175
Apr. 5	......	300	45	50	99	34	100	150	30	20	820	175
6	......	115	......	42	100	......	100	......	......	40	400	275
7	......	300	35	50	100	21	100	150	30	20	1,000	175
8 [1]	50.7	250	45	49	100	100	100	150	30	20	1,000	175
Apr. 18	......	300	45	47	100	100	100	150	30	20	1,020	175
19	......	280	45	50	100	100	100	150	30	40	1,000	350
20	......	300	45	50	100	100	100	150	30	40	840	350
21 [1]	51.1	213	30	50	100	7	100	150	30	20	1,000	175
June 5	......	300	45	35	100	100	100	150	30	20	1,000	175
6	......	275	45	50	98	2	100	150	30	20	620	175
7	......	261	45	50	100	51	100	150	30	......	1,000	......
8 [1]	52.3	276	45	55	73	18	100	150	30	40	1,000	350
Case No. 57:												
June 4	......	360	54	60	120	120	100	180	36	40	1,200	350
5	......	360	54	60	120	120	100	180	36	40	1,200	350
6	......	360	54	60	120	120	100	180	36	40	1,200	350
7 [1]	63.7	360	54	60	120	120	100	180	36	40	1,200	350
Case No. 59:												
Mar. 23	......	235	18	29	100	100	100	150	......	......	1,000	......
24	......	174	35	27	100	100	100	150	......	......	1,000	......
25	......	201	31	28	100	96	100	73	......	......	1,000	......
26 [1]	......	107	9	27	95	44	100	90	......	......	1,000	......
Apr. 5	......	200	45	22	99	40	100	83	......	......	800	......
6	......	250	45	22	100	......	100	150	......	......	1,000	......
7	......	239	45	21	100	100	100	150	......	......	1,000	......
8 [1]	......	178	45	18	100	......	100	150	30	20	1,000	175
19	......	266	45	25	100	100	100	53	30	......	820	175
20	......	300	45	43	100	43	100	150	30	......	820	175
21	......	300	45	28	100	36	100	150	30	......	1,000	......
22 [1]	......	285	45	30	100	4	......	53	30	......	1,000	......
Case No. 73:												
June 5	......	300	45	50	100	100	100	150	30	40	1,000	350
6	......	300	45	50	100	100	100	150	32	40	1,000	350
7	......	300	45	50	100	100	100	150	32	40	1,000	350
8 [1]	45.5	300	45	50	100	100	100	150	32	40	1,200	350
Case No. 77:												
Apr. 19	......	300	45	50	100	100	100	150	30	40	1,000	350
20	......	300	45	50	100	100	100	150	30	40	840	350
21 [1]	......	300	30	50	100	100	100	150	30	40	1,000	350
22 [1, 2]	56.4	300	45	50	100	100	100	150	30	40	1,000	350

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[1] Day urine was delivered.

[2] Worked 5 hours.

TABLE 9. -*Analysis of urines of patients on diet A.*

Date.	Volume.	Specific gravity.	Total acidity N/10.	Indican.	Indolacetic acid.	Total nitrogen.	Formoltitration nitrogen.	Total nitrogen.	Hippuric acid nitrogen.	Total nitrogen.	Peptid-bound nitrogen.	Total nitrogen.	Extract of autolyzed yeast.
	C. c.		*C. c.*	*Milligrams.*		*Grms.*	*Grms.*	*Per cent.*	*Gms.*	*Per cent.*	*Grms.*	*Per cent.*	*C. c.*
Case No. 1:													
Mar. 24	465	1,030	.....	0	+++	3.83	0.120	3.1	0.144	3.7	0.0	.....	.....
Apr. 7	605	1,032	111	0	+++	3.94	.120	3.0	.119	3.0	.0	.....	.....
21	415	1,029	157	0	+++	4.37	.082	1.9	.129	3.0	.0	.....	.....
June 7	930	1,017	157	?	?	5.78	.099	1.7	.129	2.2	.132	[1] 2.3	.....
Case No. 51:													
Mar. 24	2,100	1,009	.....	19	+	4.80	.110	2.3	.105	2.2	.100	2.1	.....
Apr. 6	1,705	1,011	156	36	+	5.47	.112	2.0	.....	.....	.....	.....	.....
21	3,000	1,008	124	0	0	6.16	.068	1.1	.186	3.0	.0	.....	.....
June 7	2,000	1,009	110	?	?	5.75	.082	1.4	.169	2.9	.087	1.5	.....
Case No. 52:													
Mar. 24	710	1,024	.....	Trace.	+	6.32	.113	1.8	.184	2.9	.0	.....	35
Apr. 6	580	1,030	89	0	++	4.79	.120	2.5	.171	3.6	.0	.....	35
20	635	1,026	290	Trace.	+	6.98	.148	2.1	.134	1.9	.0	.....	35
Case No. 57:													
Mar. 24	480	1,030	.....	0	+	4.99	.146	2.9	.125	2.5	.059	1.0	20
Apr. 6	505	1,032	217	Trace.	++	6.05	.160	2.6	.148	2.4	.034	.5	20
20	550	1,030	247	17	+	5.42	.160	2.9	.140	2.6	.0	.....	.....
Case No. 60:													
Mar. 10	715	1,022	.....	32	+	6.73	.174	2.6	.....	.....	.....	.....	.....
24	840	1,025	.....	15	++	6.80	.218	3.2	.142	2.1	.0	.....	.....
Apr. 7	1,350	1,018	72	27	++	5.72	.112	2.0	.....	.....	.....	.....	.....
Case No. 66:													
Mar. 23	1,460	1,014	.....	Trace.	+	6.03	.100	1.6	.054	0.9	.0	.....	.....
Apr. 6	1,060	1,016	154	0	+	5.78	.094	1.6	.....	.....	.....	.....	.....
20	1,070	1,018	190	Trace.	+	6.51	.094	1.4	.124	1.9	.03	.4	.....
Case No. 69:													
Apr. 8	635	1,039	208	14	++	5.80	.088	1.5	.159	2.7	.0	.....	.....
Case No. 70:													
Apr. 7	640	1,031	205	19	+++	6.47	.168	2.6	.187	2.9	.041	.6	50
21	680	1,028	143	18	++	5.59	.154	2.8	.161	2.9	.0	.....	50
Case No. 82:													
Apr. 23	710	1,028	114	28	+	9.34	.169	1.8	.218	2.3	.0	.....	.....
June 7	1,010	1,021	67	?	?	6.78	.187	2.8	.183	2.7	.0	.....	.....

[1] Trace of albumen in wine.

CLINICAL NOTES ON CONDITION OF PATIENTS AT ABOUT TIMES OF COLLECTING URINES.

Case No. 1 (A. L.).—Mar. 19, 1915. Sleeps well, appetite is good, is cheerful. Oral mucosa anæmia. Knee jerks normal; bowels regular.

Apr. 14. Feels strong; some dizziness; no cutaneous eruption; some burning of feet. Patient is cheerful.

June 9. Hearty, robust, no pellagrous skin lesions. Mouth normal; sleeps soundly; eats heartily. Slight tenderness on pressure of epigastrium.

Case No. 51 (M. R.).—Mar. 22, 1915. Well nourished; desquamation on hands and forearms completed. Skin slightly rough and pigmented; feet burn somewhat. Mild inflamation on mucosa of lips.

Apr. 6. Mouth not sore; mucosa of lower lip slightly congested. Burning sensation in feet at times.

Apr. 19. Irregular burning sensations over backs of hands; bowels normal. Patient cheerful; improving.

June 8. No active skin lesion visible. Forearms and neck still pigmented. Patient up and about all the time.

Case No. 52 (C. C. S.).—Mar. 18, 1915. Patient sits up and walks about; is nervous but sleeps well; appetite is good. Mild erythema over elbows; congestion of mucous membrane of mouth and pharynx; general stomatitis. Patellar reflex greatly exaggerated. Bowels normal.

Apr. 12. Feels weak and nervous; sleeps poorly; head aches occasionally; dizzy when walking; appetite is good.

Apr. 19. Mouth still slightly sore; slight redness about the elbows and slight itching on backs of hands. Mind clear; rapid coarse tremor of fingers when extended.

Case No. 57 (V. I. C.).—Mar. 29, 1915. Patellar reflex greatly exaggerated. Muscular spasms of legs, thighs, and arms.

Apr. 6. Patient sits up and walks about. Sleeps soundly. Burning of feet worse during day; appetite good; slight diarrhœa.

Apr. 19. Slight dizziness when walking. Intense burning of thighs and legs. Slight congestion of mucosa of mouth, lips, and gums. Tenderness over epigatrium. Slight swaying in Romberg position; patellar reflex still exaggerated. Confusion of mind as when admitted.

Case No. 60 (Miss E.).—Mar. 23, 1915. Desquamation over back of hands; feet and legs burn; mucosa of lips slightly inflamed. Appetite good; sleeps well; tires easily; is constipated.

Apr. 6. Backs of hands normal; tongue reddened at tip; sleeps well.

Apr. 19. Discharged, improved.

Case No. 66 (F. S. C.).—Mar. 21, 1915. On admission, erythema on backs of hands; slight desquamation over knees. No mental symptoms.

Apr. 14. Hands burn severely; feet burn at times. Mucosa of mouth still inflamed. Tires quickly but sleeps well.

Apr. 26. Lesions have almost entirely disappeared; hands about normal; tongue reddened at tip.

Apr. 30. Discharged, improved.

Case No. 69 (L. B.).—Apr. 6, 1915. Marked improvement of erythema over hands. Bowels regular; appetite good.

Case No. 70 (S. E. T.).—Apr. 2, 1915. On admission, erythema about upper end of sternum, beneath each eye, and on backs of hands. No mental symptoms.

Apr. 21. Erythema still visible on backs of hands; weakness in knees and legs. Tires quickly; patellar reflex diminished.

Case No. 82 (A. J. C.).—Apr. 20, 1915. On admission, marked erythema on backs of both hands and wrists. Mental confusion; elation; diarrhea.

June 8. Erythema has disappeared; much better mentally; slight burning in bottoms of feet. Mouth feels "drawn"; stomach feels normal; bowels move once daily; sleeps well.

TABLE 10.—*Analysis of urines of patients on diet B.*

Date.	Volume.	Specific gravity.	Total acidity N/10.	Indican.	Indolacetic acid.	Total nitrogen.	Formol-titration nitrogen.	Total nitrogen.	Hippuric-acid nitrogen.	Total nitrogen.	Peptid-bound nitrogen.	Total nitrogen.
Case No. 2:	*C. C.*		*C. C.*	*Milli-grams.*		*Grams.*	*Gram.*	*P. ct.*	*Gram.*	*P. ct.*	*Gram.*	*P. ct.*
Mar. 11	875	1,033	......	0	+	12.05	0.388	3.2	......	......	......	......
25	840	1,032	......	16	+	13.47	.272	2.0	......	......	......	......
Case No. 14:												
Mar. 25	625	1,033	......	20	+	10.84	.155	1.5	0.138	1.3	0.037	0.3
Apr. 8	795	1,031	450	26	+	12.39	.128	1.0	.144	1.2	.031	.3
June 8	725	1,029	491	?	?	11.94	.177	1.5	.106	.9	.150	1.2
Case No. 36:												
Mar. 25	930	1,030	......	63	+	13.64	.400	2.9	.134	1.0	.0	......
Apr. 8	795	1,032	474	67	++	13.05	.212	1.6	......	......	......	......
June 8	915	1,029	597	?	?	14.30	.372	2.6	.123	.8	.0	......
Case No. 48:												
Mar. 25	1,540	1,014	......	33	+	11.34	.120	1.0	.056	.5	.0	......
Apr. 8	1,295	1,017	260	38	++	11.32	.116	1.0	......	......	......	......
21	1,400	1,018	380	80	++	13.45	.102	.7	.159	1.2	.0	......
June 8	1,635	1,013	174	?	?	10.27	.130	1.3	.169	1.6	.063	.6
Case No. 57:												
June 8	1,120	1,026	456	?	?	13.15	.209	1.6	.152	1.1	.161	1.2
Case No. 59:												
Mar. 26	845	1,019	......	21	0	6.91	.128	1.8	......	......	......	......
Apr. 8	485	1,029	304	30	0	7.10	.148	2.1	......	......	......	......
22	1,030	1,019	285	36	0	9.02	.114	1.3	.065	.7	.012	.1
Case No. 73:												
Apr. 22	[1] 1,600	1,021	258	78	+	8.25	.365	4.4	.039	.47	.0	......
June 8	1,610	1,016	248	?	?	9.09	.112	1.2	.170	1.9	.0	......
Case No. 77:												
Apr. 21 [2]	1,022	1,027	435	10	+	11.97	.086	.72	.125	1.0	.01	.08
22 [3]	1,022	1,022	414	23	+	12.02	.088	.72	.155	1.3	.031	.25

[1] On diet C, consisting exclusively of milk, eggs, and bread.

[2] Resting.

[3] Worked at carpentry 5 hours.

CLINICAL NOTES ON THE CONDITION OF PATIENTS AT ABOUT TIMES OF COLLECTING URINES.

Case No. 2 (G. O. C.)—Mar. 23, 1915. Sits up, walks about, some dizziness, slight erythema on back of both hands and elbows. Tingling sensation on back of hands; tongue slightly red at the tip; bowels move daily. Mucosa of lower lip slightly reddened.

Case No. 14 (W. A. McD.).—Mar. 18, 1915. Skin on dorsum of hand glazed, cuticle atrophied, mild erythema. Burning sensations in backs of hands, burning over soles of feet severe at night. Walks out, but tires easily.

Apr. 3. No change.

Apr. 19. Burning sensations improved; appetite good.

June 5. Skin of hands smooth; tongue slightly reddened.

Case No. 36 (W. J. McK.).—Mar. 18, 1915. Dorsal surfaces of hands smooth. Burning sensations persistent over plantar surfaces of feet. Patellar reflex exaggerated. No mental symptoms. Loose stools daily.

Apr. 9. Gaining in weight; sleeps well. Tongue normal.

Apr. 19. Slight erythema (new) over backs of hands; feet burn; bowels normal.

June 5. General condition good: slight desquamation on right hand. No burning of hands.

Case No. 48 (M. S.).—Mar. 22, 1915. Vertigo, but no mental symptoms. Asthmatic symptoms.

Apr. 6. Improved; sleeps well; has burning sensations, but no erythema on neck, scalp, feet, and hands.

Apr. 19. Has severe burning of mouth, throat, and stomach, also of feet and legs. Slight diarrhea.

June 8. Still has burning sensations in hands. Face is flushed: tongue fissured, tip clean and smooth. Patellar reflex markedly exaggerated. Up and about the ward. Patient is nervous and cries frequently.

Case No. 57 (V. I. C.).—June 5, 1915. Skin on hands smooth. Tongue slightly reddened at tip. Burning of feet, legs, and thighs; some weakness in lumbar region; patellar reflex markedly exaggerated, ankle clonus present.

Case No. 59 (E. S.).—Mar. 23, 1915. Confined to bed, very weak, lesion over knuckles and fingers. Mucosa of mouth anemic; tongue anemic, but clean.

Apr. 6. Sits up; no dizziness; sleeps well. Burning sensations in feet. Tongue normal. Appetite good; is cheerful.

Apr. 21. Gaining weight; sleeps well.

Case No. 73 (J. G. H.).—June 5, 1915. Sitting up, but feels very weak. Skin of hands soft and smooth. Patellar reflex greatly exaggerated; weakness in legs, marked in right foot and leg.

Case No. 77 (J. J. F.).—April 20–21, 1915. Patient improved; walks about, has good appetite. Slight burning on soles of feet.

It may be noted that many of the patients during the course of these observations showed marked improvement. For example, the skin lesions cleared up, the mouth became well, the burning sensations were mitigated, the diarrhea disappeared, the appetite increased, etc. Yet the most careful scrutiny of these details fails to reveal any definite correlation between the clinical condition and the laboratory findings. The number of patients which could be handled under perfect control in the time at my disposal was too small, however, to permit of a definite negative conclusion in the matter. What I feel reasonably certain of is that if any such marked improvement were due to a sharp alteration in protein metabolism involving the functions of digestion, absorption, deamination or excretion, the coincidence would have been detected.

A few changes in the amount of formol-titration nitrogen excreted, however, require brief comment. With patients Nos. 1, 51, and 60 (Table 9), the urine at the earlier examinations contained a noticeably higher amount of this fraction, both relatively and absolutely, than later. The same tendency was observed also in a few other patients and possibly would have been more evident had not the yeast extract been given. When this difference was first observed, it was the writer's expectation that a marked improvement in the gastric condition of the patients would be noted. Mr. Givens states, however, that no such change is detectable in his numerous examinations of the stomach contents. We shall see presently that as between different patients in these groups, taking averages of urinary and gastric analyses, in agreement with the difference already observed between patients Nos. 81 and 83 (pp. 58, 59), there is some indication that the amount of formol-titration nitrogen excreted is related to gastric condition; but it is necessary to conclude that the amount of this fraction may, on the other hand, undergo a very noticeable change without a corresponding improvement in the gastric function.

It is possible, nevertheless, that there was better utilization of food resulting from better adaptation to the diet as time went on. The burden of digestion in patients with low free gastric acidity and with low pepsin number must obviously fall upon the pancreas, and it would not be surprising if, with the rest and regular dietary habits of the hospital, intestinal digestion would be improved. This would result in more complete cleavage of the protein material and consequently better deamination and utilization beyond the intestinal wall.

A mild test of the capacity of these patients to handle ultimate cleavage products was afforded, quite unintentionally, by the administration to three of them (Nos. 52, 57, and 82) of the concentrated extract of autolyzed yeast.[1] This material was being given by Dr. Voegtlin for the purpose of supplying so-called vitamines, the deficiency of which in the food, according to the hypothesis of Funk (5), is the cause of the disease. It was found upon subjecting the extract to analysis that about 40 per cent of the biuret-free nitrogen was in the form of monamino acids. Hence a comparison of the excretion of formol-titration nitrogen following the administration of the yeast extract would afford proof, if there were no increase, that the deamination of such bodies was properly performed.

In 10 c. c. of the original concentrated extract were found: Total nitrogen, 0.379 gram; formol-titration nitrogen in filtrate from phosphotungstic acid precipitate and removal of phosphotungstic acid by $Ba(OH)_2$, 0.150 gram. The formol-titration nitrogen in 20 c. c. of such extract would correspond, therefore, to 1.6 grams glycocoll, or 1.9 grams alanin. In 50 c. c. there would be the equivalent of 3.9 grams glycocoll, or 4.8 grams alanin. This, obviously, is not a severe test of the deaminating function or of the capacity of the tissues to fix this form of nitrogen, and the absence of any abnormal quantity in the urine following its administration signifies only that the liver and other tissues were perfectly capable of handling free amino acids in this quantity in a perfectly normal fashion, notwithstanding that their absorption would probably take place in a very short time after the material reached the intestine. It must be borne in mind in this connection that the tissues of the patients maintained on diet A. would be in nowise surfeited with nitrogenous split products, and it is therefore possible that the extra supply was very promptly withdrawn from the circulation by very needy cells. No more searching test of the capacity to handle the split products could, because of the scarity of the material, be made.

What has been said with reference to the formol-titration nitrogen of the urine, in relation to improvement in clinical condition, applies equally to the other fractions, namely, that no definite correlation can be made out.

AMINO FRACTIONS IN RELATION TO THE GASTRIC CONDITION OF DIFFERENT PATIENTS.

Through the courtesy of Mr. Givens, the writer is able to present in table 11 the average figures obtained by him, in most of the patients used in this study, from the stomach contents taken on the same days that the urine was obtained. In the same table are brought together the average figures for the total acidity of the urine, indican, formol-titration nitrogen, total nitrogen, and the maximum yield of peptid-bound nitrogen. The table does not require an extended discussion, for the reason that no definite parallelism exists between gastric conditions and urinary results. For both dietary groups one can say only that the lowest amino-acid fraction (percentage) is found in patients who have a satisfactory gastric condition, and the highest is found in patients who have a poor gastric condition. In the intermediate conditions there appear to be exceptions both ways, as well as cases which appear to obey such a rule. All one would care to hazard in the way of generalization is that there is a tendency for high formol-titration nitrogen to coincide with poor gastric condition and vice versa. It is regrettable that time did not suffice for following up more cases with this comparison in mind.

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[1] This extract represents the alcohol soluble fraction of autolyzed brewer's yeast

TABLE 11.—*Comparison of the amino fractions in the urine with the gastric condition.*

Case No.	Character of case.	Gastric condition. (average).			Urine.					
		Total acidity N/10.	Free acidity N/10.	Pepsin No.	Total acidity N/10.	Indican (daily average).	Formol-titration nitrogen (daily average).	Total nitrogen (daily average).	Formol-titration nitrogen (daily average).	Peptid-bound nitrogen (largest amount found).
	DIET A.					*Milligrams.*	*Grams.*	*Grams.*	*Per cent.*	*Grams.*
1	First attack	74- 84	52-70	21-31	134	0	0. 107	4. 03	2. 6	0. 132
51	.....do	40- 80	12-41	8-10	140	19-36	.096	5. 44	1. 8	. 100
52	Recurrence, moderately severe	80- 90	60-86	21-31	290	0	. 127	6. 03	2. 1	. 0
57	.....do	10- 28	0	0	237	0	. 155	5. 48	2. 8	. 059
60	First attack, mild	56- 61	21-40	16-21	72	15-32	. 168	6. 41	2. 6	. 0
66	Recurrence, mild	80-120	60-92	26-41	170	0	.096	6. 10	1. 6	. 030
70	First attack, modererately severe	60- 71	40-50	8-16	174	19	. 161	5. 88	2. 7	. 041
	DIET B.									
2	Recurrence, moderate.	21- 24	0	Tr. 10	....	16	. 330	12. 5	2. 6	....
14	Severe	32- 40	3- 7	Tr. 12	450	23	. 153	11. 7	1. 3	. 150
36	Recurrence, severe	32- 50	3- 5	Tr. 10	474	65	. 328	13. 6	2. 4	. 0
48	Recurrence, moderately severe	14- 30	0	Tr. 8	320	50	. 117	11. 6	1. 0	. 063
57	.....do	10- 28	0	0	456	....	. 209	13. 1	1. 6	. 161
59	First attack, mild	14- 45	0- 7	Tr. 20	315	30	. 130	7. 7	1. 7	. 012
77	.....do	64- 96	41-74	12-21	424	15	. 087	12. 0	0. 7	. 031
	DIET C.									
73	First attack, severe	27	41-74	0	258	78	. 365	8. 25	4. 4	. 0

Only one case (No. 73) exhibits an amount of formol-titration nitrogen which could be called distinctly abnormal. This patient was very ill at the time and was being maintained on an emergency diet of milk, cream, and eggs, a diet which itself might in part at least be responsible for the analytical findings (see p. 48). The gastric condition of this patient was very bad.

COMPARISON OF URINES OF PELLAGRINS WITH THOSE OF NORMAL PERSONS.

Taking as normal the limits arrived at from a study of the literature (p. 47), it is clear that all the results in the amino-acid fractions, with the single exception just mentioned, may be regarded as normal. We reach the same conclusion with regard to these fractions as have Meyers and Fine (8) with regard to the urea, ammonia, creatinin and uric acid fractions of the total nitrogen.

Looking at the results for hippuric acid, however, it is evident that we have in these subjects an unusual condition. The amount of hippuric acid nitrogen is distinctly higher than any hitherto reported in normal persons on an average mixed diet, and, in fact, is about the amount reported by Chittenden and by Herter (32) for subjects receiving as much as two grams of sodium benzoate daily. For better emphasis of this point there are brought together in Table 12 the results obtained on 15 pellagrous subjects with those obtained by Herter and Chittenden on nine normal healthy young men.

No question remains regarding the adequacy of the method employed in the investigation. It has been checked both before the work was started and since it has terminated, and there is no doubt that, with sufficient care as to the end point in the titration, it gives reliable results (p. 56).

TABLE 12.—*Comparison of hippuric acid excreted by normal men and by pellagrins.*

Normal men.			Pellagrins.		
Subject.	Total nitrogen.	Hippuric acid nitrogen.	Subject.	Total nitrogen.	Hippuric acid nitrogen.
REPORTED BY CHITTENDEN.			DIET A.		
H. H. G	12.59	0.064	A. L	4.48	0.131
	8.53	.037	M. R	5.80	.153
W. W. H	12.59	.054	C. C. S	6.03	.163
	8.35	.023	V. I. C	5.48	.137
L. M. L	12.11	.051	F. S. C	6.51	.124
	9.43	.027	L. B	5.80	.159
J. F. L	10.39	.046	S. E. T	5.98	.174
	10.01	.038	A. J. C	8.06	.200
E. C. M	12.46	.046	Average	6.02	[3].155
	9.83	.054			
W. C. R	9.93	.054	DIET B.		
	8.58	.048			
Average	10.4	[1].044	W. A. McD	11.69	.129
REPORTED BY HERTER.			W. J. McK	13.99	.128
			M. S	13.97	.128
			V. I. C	13.15	.152
I. R	9.19	.06	E. S	9.02	.065
	9.23	.05	J. G. H	9.09	.170
	8.82	.05	J. J. F	11.99	.140
H. H	15.6	.09	Average	11.54	[4].130
	16.4	.08			
	12.8	.06			
	15.4	.08			
H. O	12.7	.07			
	12.8	.07			
Average	12.57	[2].07			

[1] 0.044=0.44 per cent. [2] 0.07=0.55 per cent. [3] 0.155=2.5 per cent. [4] 0.130=1.1 per cent.

From this comparison it is evident that the excretion of hippuric acid by pellagrins may amount to as much as four times the quantity usually excreted by healthy men on ordinary mixed diet. With due allowance made for the difference in methods employed (p. 55), there still remains a considerable excess—probably two or three times the usual elimination.

We have already seen (p. 7) that the dietary habits of the people afflicted with the disease in this country at least conform in a general way to the dietary conditions which are necessary for the production of this chemical body in man. Pellagrins ingest a disproportionate amount of vegetable and farinaceous material, with plentiful quantities of sugar, and in some cases the intestine is the seat of an unusual degree of putrefaction. It is noteworthy that the patients on diet A, which was chosen by Dr. Voegtlin to represent as nearly as might be the diet habitually consumed by these people at their homes, excreted considerably more hippuric acid than those on a diet containing plenty of animal protein.

Whether this condition of the intestine, with the habitual production of benzoic acid in quantities which the human organism in temperate climates is scarcely accustomed to, can be of any significance in the etiology of the disease is a question which requires more detailed study. One could readily imagine that a chronic intestinal condition of this kind might lead, possibly through deprivation of the body of its store of glycocoll, possibly through toxic effects, to serious consequences. The fact that hippuric acid is found as a constituent of the epidermal scales in certain other skin diseases, e. g., ichthyosis, may be significant.

That such quantities of benzoic acid as are here reported (as hippuric acid) can not be immediately harmful is witnessed by the fact that many of the patients on diet A were showing marked improvement while the urines were being collected, as well as by the fact that the Remsen Board (U. S. Department of Agriculture, Report No. 88) found no harmful effects from the administration of 2 grams (and more) of sodium benzoate daily for several weeks. But pellagra is not a disease which develops in a few weeks. From all indications it is the outcome of chronic conditions.[1]

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[1] Since the foregoing was written, I have read the contribution of Lorenz on the "Mental Manifestations of Pellagra" (47). He has reached the conclusion that there are many points of resemblance between the effects of this disease and those of alcoholism, and suggests that the etiological factor may be a form of intoxication from "a substance of chemical nature similar to alcohol, in that it has specific deleterious properties."

It is not yet known what bodies other than benzoic acid, phenaceturic, etc., may arise under the conditions above specified. Indeed, the formation of alcohol itself by fermentation of carbohydrate, in perfect analogy to the commercial preparation of alcohol in the form of whisky from "sour mash," even in the intestinal tract of man, is by no means excluded, especially in the absence of hydrochloric acid in the stomach.

CONCLUSIONS.

Two points of possible significance may be restated in conclusion:

(1) It is clearly suggested, by comparison of two subjects, one without free acid and pepsin in the stomach and the other with normal gastric juice, that the quantity of formol-titration nitrogen in the urine may depend on gastric condition, and this is borne out to some extent by other cases. Since this implies imperfect cleavage of protein in the absence of good gastric digestion, toxic effects may result.

(2) The quantity of hippuric acid excreted by pellagrins, especially those kept on a corn-vegetable diet, is from two to three times the quantity excreted by normal men on a general mixed diet. The question may be raised whether this does not denote an intestinal condition capable of producing toxins which may have far-reaching somatic effects.

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IV. THE OCCURRENCE OF PELLAGRA IN NURSING INFANTS, WITH OBSERVATIONS ON THE CHEMICAL COMPOSITION OF THE HUMAN MILK FROM PELLAGROUS MOTHERS.

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PREVIOUS LITERATURE.

Pellagra occurs only rarely in nursing infants, but undoubtedly the disease does sometimes appear during the first two years of childhood. The literature on pellagra contains a few brief references to this subject.

Thus Strambio, as early as 1794, writes, "As to age, I have already published many histories of pellagrous children, not only under 12 years of age, but 6, 4, 2, or even *sucklings*." (Italics ours.)

Sambon (1910) describes a case of undoubted pellagra in an infant seven months old.

Roberts (1914) states that the youngest cases which he found were in infants four or five months old.

Wood (1912) states that his youngest pellagrin was 22 months old.

Snyder (1912) reports the results of an inquiry into the occurrence of pellagra in children in the United States. This writer sent out cards to physicians, with the request that they report any case which had come under their observation. The youngest case reported was an infant two months old, who had nursed from his pellagrous mother for four weeks. The child was then fed on a proprietary milk preparation. The mother died of pellagra when the infant was six weeks old. "The erythematous rash did not appear until several days after the mother's death, but I was informed by the midwife, who attended the mother, that the infant was much emaciated at birth, and had been affected with profuse, foul-smelling diarrhœa since one week old." No further details of this case are given.

In Lavinder and Babcock's translation of Marie's Pellagra (1912) the following statement is made: "Most authors agree that the disease does not occur in infants unless they are fed on spoiled corn."

Weston (1914), writing on this subject, reports a case of pellagra in an infant five and one-half months old. This patient was breast fed until shortly before the pellagrous symptoms appeared. Severe diarrhœa, a reddened tongue, a symmetrical rash over both feet and on the ankles, and a discoloration on the dorsal surface of the hands were observed. A few days before the erythema appeared the infant was put on rice water, which was followed by casein milk.[1] "Both parents were strong and healthy. There was no history in any other member of the family and the child was entirely breast fed."

Perhaps the most instructive and best description of a pellagrous infant is found in a paper by Merk (1906). This considered the clinical aspects of the case only.

Case observed by the writers.

In the cases referred to above only casual attention was paid by these authors to the diet of either mother or child. It may not be out of place, therefore, to describe here a most interesting case of pellagra in a nursing infant which came to the writer's attention.

Case report.

Personal history.—The infant, white female, was born May, 1914. It had never been fat and well nourished, although perfectly healthy from the time of birth until the age of 5 months (September, 1914), when it was noticed that its mouth became sore. Up to this time the child had been breast fed. The mouth continued sore during the winter of 1914–15. At the end of May, 1915, the infant developed an intense diarrhœa. During the winter of 1914–15 the mother continued nursing the child, who was also fed, in addition, some corn bread and a half teacup of buttermilk daily. Sometimes beans, biscuits, or a little rice were given to the infant. In June, 1915, an extensive erythema appeared, involving the whole face, hands, and legs. The child, then at the age of 14 months, was brought to the Pellagra Hospital by her parents and the family physician, July 15, 1915.

Family history.—The father of the child (43 years old), and the mother (29 years old), on being questioned denied ever having had a dermatitis suggesting pellagra, and neither exhibited at this time any definite pellagrous symptoms. The mother, however, complained of chronic indigestion and was obviously undernourished. The other children of the family, aged 13, 11, 8, and 3 years, respectively, were healthy, according to the statements of the family physician. This was verified later on by a visit to the home of the family. The occupation was farming and the principal product cotton. The family owned a cow; the milk was used by its members and butter made therefrom at home. About a dozen hens were kept, but the eggs were often sold. No hogs were kept. Some corn, beans, cabbage, beets, peas, onions, and tomatoes were raised. The family seldom bought beef, because it was difficult to obtain. Homemade molasses was used, mainly during the winter season, and white rice, highly milled wheat flour, and cottonseed lard were bought at a near-by store. Corn meal was milled in a local, old-fashioned mill from home-raised corn. The mother of the infant was very fond of corn bread and vegetables, and was accustomed to drinking about two glasses of buttermilk for supper, but none with other meals. She did not care for eggs. She ate string beans in the summer, but no beans of any sort during the winter. No fresh meat was eaten by her at any time.

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[1] By casein milk is probably meant a protein rich milk, advocated by Finkelstein and Meyer for the treatment of so-called decomposition in infants. The milk is prepared by mixing the curds of 1 quart of whole milk with 1 pint of buttermilk (from skimmed milk) and adding enough water to bring the volume up to a quart.

Physical examination.—July 15, 1915. An erythema involved the dorsum of both hands and feet, and was symmetrical in distribution. Desquamation had begun. The face also showed erythematous areas. The mouth and tongue were reddened and the mucous membrane inflamed. The patient was anemic and had a poorly nourished appearance. The mother stated that the child had diarrhea. The infant was nursed at breast while seen in the hospital. According to the mother the child ate at that time with great difficulty, was fretful, and cried a great deal. The diagnosis of pellagra was confirmed by Passed Asst. Surg. Grimm.

Subsequent history.—On October 13, 1915, the child was again seen at its home in Spartanburg County, S. C., where the family had been living for a long time.

The information obtained on July 15 was confirmed. The father was not seen, but his wife stated that he was well. The mother and the children other than the patient were free from any pellagrous symptoms. The mother stated that she was still troubled with indigestion and that she also vomited occasionally after eating. She complained of heartburn and sour stomach. She gave a history of having had indigestion for the last 10 years, although this had improved during the last summer. Her average weight had been 112 to 119 pounds; she now weighed 140 pounds. No stomatitis, dermatitis, or other pellagrous signs were observed in the mother. The four other children were healthy and well nourished.

Examination of the child showed a brownish erythematous area over the nose and cheeks. The skin of the dorsum of the hands was wrinkled, atrophied, and thickened. On the dorsum of the feet there was noticed a subsiding pellagrous dermatitis and the skin was peeling off. The mouth was normal, except the tongue, which was still bright red and denuded of epithelium. The mother stated that the baby's condition grew worse after her visit to the hospital; the diarrhoea increased and the stools became green in color at times. The skin of the face, however, had cleared up until about two weeks previously, when a fresh erythema appeared.

The baby was still nursed at the breast and received, in addition, some buttermilk and wheat biscuits. Occasionally a soft-boiled egg and bean soup had been given, which seemed to give rise to colic. The child did not take much buttermilk at that time, but ate biscuits every day. The biscuits were prepared from highly milled wheat flour, soda, and buttermilk. The child refused sweet milk. The mother stated that she had had enough breast milk for the baby until recently.

On June 3, 1916, the family was again seen at the farm. The infant had died on February 24 of this year. According to the parents' statement the patient's condition had gradually improved with the beginning of colder season (October, 1915). The child had gained considerably in weight (from 11 pounds in September, 1915, to 20 pounds in February, 1916). The skin symptoms had cleared up entirely, and the tongue had become normal in appearance. The diarrhoea also had improved considerably. During the last week in February, 1916, the patient developed what appears to have been pneumonia. The child had fever for two days and died rather suddenly. The family physician stated that death was due to "pulmonary congestion." The mother stated that the infant was nursed at her breast up to the time of death; whenever she tried to wean the baby the diarrhoea increased in severity. She, therefore, had kept the child at the breast and fed it, as formerly, a little buttermilk, biscuit, corn bread, and vegetables from the table in addition.

None of the members of the family showed any pellagrous symptoms at this time, and all stated that they were feeling well.

This case is of special interest for several reasons:

From a careful perusal of the literature this seems to be the first record of pellagra occurring in a nursing infant whose mother and other members of the same family were apparently free from the disease.

There is no doubt that the patient was receiving breast milk throughout its life. It should be emphasized, however, that the patient also received some other food besides the breast milk (corn bread, wheat biscuits, buttermilk, and some fresh vegetables).

Several other cases of pellagrous infants have come to our notice, but in these latter cases the infants were always nursed by their pellagrous mothers.

TOPIC OF PRESENT INVESTIGATION.

From the point of view of the dietary theory of pellagra these cases are of great interest, inasmuch as milk is very probably the most efficient therapeutic and prophylactic food known. Analysis of the causes underlying the appearance of pellagra in breast-fed infants seems, therefore, of great value in elucidating the purely scientific aspects of the etiology of this disease. The dietary problem of such cases limits itself largely to one definite food whose normal composition is pretty well known. If pellagra be due simply to an abnormality of the diet, it should be possible to detect the defect of breast milk which has caused the disease in infants. Several possible explanations of this problem suggest themselves.

(1) It can be conceived that a one-sided diet of a nursing mother might lead to an alteration of the chemical composition of her milk, affecting mainly the known milk constituents (protein, carbohydrate, fat, and inorganic salts). It may be emphasized in this place, however, that all previous work on the relation of diet to milk secretion has shown that it is very difficult to change the chemical composition of milk by changes in diet. The mammary gland tends to keep the chemical composition of the milk constant, within relatively narrow limits. Thus Holt states that marked changes in the protein and fat content of the diet of lactating mothers may lead to only minor fluctuations in the protein and fat content of human milk. The sugar content also varies but little. The milk fat and protein are increased slightly by overfeeding with fats and carbohydrates, and reduced somewhat by a considerable elimination of these foods from the dietary. An increased intake of water stimulates the secretion of milk. Starvation lowers the fat and sometimes the protein. No observations are on record where a change in diet has led to changes in the qualitative composition of the milk constituents of known composition.

(2) In the absence of any considerable quantitative changes in the constituents of milk derived from pellagrins, the possibility still remains that there might occur changes in the constituents of unknown chemical composition, namely, the so-called vitamines. Changes of this kind could not, of course, be demonstrated by chemical analysis, as no such methods are available for this purpose. The only method for the detection of a lack of vitamines is the biological test, namely, the feeding of the milk to animals on a deficient diet.

(3) Another cause of the pellagrogenic properties of human milk might, perhaps, be found in some toxic substances which are secreted with the milk of pellagrous mothers. This interpretation seems, however, to possess very little in its favor, as will be seen later. It might suffice here to state that the presence of toxic substances has never been demonstrated in the milk, blood, or tissues of pellagrins.

As a beginning of studies of this kind, the writers decided to analyze, by means of the ordinary analytical methods, milk obtained from pellagrins.

EXPERIMENTAL PART.

The milk was obtained from patients who were treated at the Pellagra Hospital of the United States Public Health Service at Spartanburg, S. C. The patients, after admission, were put for a certain period of observation on a diet very similar in composition to the diet which seemed to be consumed by the patient prior to the attack with pellagra. This diet is designated as diet A and consists of the following articles. (For chemical composition see Table 2.)

Breakfast.—Hominy, 75 grams; butter, 15 grams; corn sirup, 30 grams; wheat bread, 100 grams; coffee, with 20 grams sugar and 20 c. c. milk.

Dinner.—Potatoes, 150 grams; cabbage, 50 grams; turnip tops, 50 grams; fat pork, 35 grams; wheat bread, 100 grams.

Supper.—Corn meal mush, fried in pork fat, 50 grams; lean boiled ham, 25 grams; prunes, 30 grams; wheat bread, 100 grams; coffee, with 20 grams sugar and 20 c. c. of milk.

After a shorter or longer time, the diet was changed to the following, designated as diet B. (For composition see Table 3.)

Breakfast.—Eggs, 100 grams; milk, 180 c. c.; butter, 15 grams; wheat bread, 100 grams; orange juice, 100 c. c.; coffee, 50 c. c., with 20 grams of sugar and 20 c. c. of milk.

Dinner.—Lean beef, 100 grams; potatoes, 150 grams; butter, 15 grams; milk, 200 c. c.

Supper.—Corn-meal mush (fried in 15 grams pork fat), 50 grams; coffee plus 20 grams sugar and 20 c. c. of milk; prunes, 30 grams.

At 8 p. m. 200 c. c. milk.

Diet A is mainly vegetable in character, low in protein and high in carbohydrate content. Diet B is rich in animal protein and possesses a high nutritive value and is rich in accessory foods usually called vitamines.

The writers have been unable to find any data referring to the influence of diets of the above character on the composition of human milk.

All of the five patients whose milk was analyzed had uncomplicated cases of pellagra, two of them being of a very severe type. The milk was obtained while the patients were on diet A in all cases. In one case a yeast extract was later on superimposed on diet A. In two cases the diet was changed to B, and the milk obtained during this period was also analyzed.

The milk samples were obtained by the nurse massaging the breast of the mother four times daily—at 6.30 a. m., 11 a. m., 4.30 p. m., and 9 p. m. Each sample was brought at once to the laboratory and placed in a bottle containing sufficient formaldehyde to preserve the milk. The samples were kept in an ice box until the time of analysis.

Methods employed for making analysis.

Nitrogen.—Total nitrogen was determined by the Kjeedahl-Gunning method.

Total protein nitrogen[1] was determined by the Liebermann-Sebelien method. The proteins are precipitated by tannin, acetic acid, and alcohol in sodium chloride solution.

Nonprotein nitrogen[1] was determined in the above filtrate.

Casein[1] was determined by the Schmidt modification of the Hoppe-Seyler method.

Albumin[1] was obtained from the filtrate of the above method.

Fat.—The fat was determined in the dried material, by extraction with anhydrous ether according to a modification of the Soxhlet method.

Sugar.—The sugar was determined by titration with Benedict's solution, after the precipitation of the proteins with colloidal iron, as described by Hill (1915).

Total solids.—The total solids were determined by drying 10 cubic centimeters of milk in a flat dish, on a water bath, for three hours. The dish was then placed in an electric oven at 80° C. for 30 minutes, cooled, and weighed.

Total ash.—The dried sample was carefully charred by gentle heating, and extracted with hot water. The residue was completely ashed, and the washings were evaporated to dryness. The dish was heated gently, cooled, and weighed.

Chlorides.—The milk was ashed with potassium nitrate mixture, and the chlorides were titrated according to Volhard's method.

Phosphorus.—The phosphorus was determined by titration. Ten cubic centimeters of milk were ashed by Neumann's method and the phosphorus was precipitated with ammonium molybdate solution. The precipitate was dissolved and titrated with N/14 sodium hydroxide.

Calcium, magnesium, potassium, and sodium.—These constituents were determined in the same portion. One hundred cubic centimeters of milk were ashed by Neumann's method. Calcium was precipitated as oxalate, heated to oxide, and titrated with acid. Magnesium was precipitated as magnesium ammonium phosphate. This precipitate was converted into ammonium phosphomolybdate and titrated with sodium hydroxide. Potassium and sodium were weighed as chlorides. The potassium was precipitated with sodium cobaltic-nitrite and titrated with permanganate solution. This method is described in detail by Lewis (see p. 38).

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[1] See Abderhalden "Handbuch der Biochemischen Arbeitsmethoden," pp. 447–450.

METHODS EMPLOYED FOR THE ANALYSIS OF FOOD OF PATIENTS.

Moisture.—The sample was dried in vacuum desiccators over phosphorus pentoxide at 60 to 70° C. to constant weight.

Ash.—The sample was ignited at low temperature and the carbonaceous mass was extracted with water. The residue was ashed. The water extract was added to the ash and evaporated to dryness. The dish was gently heated and weighed after cooling.

Protein.—The total nitrogen was determined and was multiplied by the following factors, which are generally employed in work of this nature:

Wheat	5.70
Corn	6.39
Other foods	6.25

Fat.—The water-free sample was extracted with anhydrous ether for three days. The extract was dried to constant weight. Some samples, after this extraction, were saponified with alcoholic KOH, acidified with HCl, and extracted with ether. This extract was added to the original extract.

Carbohydrate.—Determined by difference. (Total caloric value of food minus caloric value derived from fat and protein.)

Calories.—The sample was ignited in a Riche adiabatic calorimeter which had been standardized against sugar and benzoic acid from the Bureau of Standards. The caloric value was also calculated from the percentage composition with the following factors:

Protein	5.7
Fat	9.3
Carbohydrate	4.1

The physiologic value was calculated from the percentage composition, as above, using the factor of 4.1 for protein instead of 5.7.

Brief description of patients.[1]

Case No. 55.—Admitted February 20, 1915; discharged March 27, 1915. White, female, aged 28 years, married. Seven months old baby boy is admitted with mother.

Mother has pellegra for several years (first attack spring of 1909). Lived in cotton-mill village. On admission mother shows marked symptoms of pellagra—pigmented dermatitis, involving dorsum of both hands and lower foreams, increased reflexes, mental confusion, vertigo. Is confined to bed most of the time; feels weak, mild stomatitis, diarrhœa, appetite fairly good, suppressed menstruation since birth of last child.

Infant is nursed at breast, but mother seems to have very little milk, therefore, child is fed modified cows' milk. Child has undernourished appearance. Mother states that she fed child on turnip salad, potatoes, etc.; in fact, just anything she ate herself, besides nursing it at the breast. The infant does not present any definite pellagrous symptoms, although mother has severe attack of the disease. Diet A was given throughout paoient's stay in the hospital. Discharged only slightly improved. Patient gave only a small volume of milk on massaging the breast. Specific gravity of milk varied between 1033 and 1034. Total daily volume from 100 to 200 c. c.

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[1] These notes are taken from the histories prepared by Drs. Grimm and Allen.

Case No. 68.—Admitted March 25, 1915; discharged April 26, 1915. White, female, aged 32 years, married. Did cotton-mill and house work. Youngest child 10 months old; is healthy, according to mother's statement; normal labor.

First pellagrous attack in June, 1914. Present attack appeared about March 1, 1915. Weakness, sore mouth, redness of hands and diarrhœa. On admission she is considerably undernourished, has stomatitis, pellagrous skin lesions over dorsum of both hands and elbows. Slight desquamation over forehead. No pronounced mental symptoms. Reflexes exaggerated, bowels loose; is able to sit up and walk about during the day; burning of feet; feels fairly well. Body weight 39.5 kilos.

Fair amount of milk is obtained by massaging patient's breasts. Diet A given throughout stay in hospital. Discharged considerably improved. The breasts of this patient were massaged regularly at 6.30 and 11 a. m., and 5 and 9 p. m. The volume of milk obtained was as follows:

	Cubic centimeters.		Cubic centimeters.
Mar. 26–27	771	Apr. 10–11	671
27–28	540	11–12	672
28–29	564	12–13	667
29–30	600	13–14	659
30–31	650	14–15	598
31–Apr. 1	610	15–16	646
Apr. 1–2	690	16–17	591
2–3	610	17–18	626
3–4	720	18–19	554
4–5	632	19–20	513
5–6	719	20–21	538
6–7	640	21–22	499
7–8	684	22–23	447
8–9	720	23–24	434
9–10	680	24–25	422

Case No. 80.—Admitted, together with her 10-months-old child, April 19, 1915; discharged May 30, 1915. White, female, aged 26, married; has four children.

First pellagrous attack March, 1914. Present attack March, 1915, with stomatitis and erythema. Complains of burning of feet, weakness, abdominal pains, dizziness; nourishment deficient; pale and anemia. Inflammation of gums and of tongue, which is denuded. Slight erythema over knuckles, dorsal surface of thumbs, extensor surface of wrists, and over lower portion of flexor surface of forearms. Sleeps very poorly, is constipated, appears depressed; appetite good; patellar reflexes exaggerated. Menses have not appeared since birth of last child. Hemoglobin, 85 (Sahli scale). Patient was on diet A from April 20 to May 9, then on diet B. She gradually improved.

The 10-months-old infant was weaned, after admission to the hospital, and fed on modified cows' milk, on which he seemed to get along well. Patient discharged considerably improved. Breast feeding of infant was stopped April

26, 1915. Milk obtained by massaging breasts four times daily. Daily volume of milk was as follows:

	Cubic centimeters.
Apr. 29–30	354
30–31	230
May 1–2	279
2–3	331
3–4	456
4–5	398
5–6	444
6–7	379
7–8	338
8–9	394
May 9–10	290
10–11	330
11–12	310
12–13	316
13–14	308
14–15	229
15–16	246
16–17	200
18–19	119
25–26	118

Case No. 86.—Admitted May 14, 1915; discharged August 1, 1915. White, female, aged 26, married, farmer's wife. Has four children living; two dead. No other pellagrins in family. Youngest child five and one-half months old.

Pellagrous symptoms appeared in February, 1915. Had sore mouth, erythema of hands and diarrhœa. Complains of intense burning of the feet, swelling of feet and legs, great weakness, and sore mouth. There is a pellagrous skin lesion over the dorsum of both hands, fingers, wrists, dorsum of feet and cheeks; no mental symptoms. Appetite poor; absence of menses since birth of last child. Appears fairly well nourished, slightly anæmic; mucosa of mouth reddened, tongue fissured and reddened. Erythema over dorsum of both hands and wrists, with pigmentation; elbows pigmented and rough; erythematous patches over cheeks and dorsal surfaces of feet; feet slightly swollen. Patellar reflexes increased on both sides. No marked mental symptoms; sleeps poorly. Hæmoglobin, 80 (Sahli scale). Vomits at times. Diet A was given throughout; this was increased by 1/5, June 27. May 30 to July 4, patient received an alcoholic extract from autolyzed yeast (vitamine). Patient's condition gradually improved, especially after the yeast preparation was added to diet. Patient slept better, diarrhœa disappeared, skin lesions healed, and patient left the hospital without any obvious symptoms of pellagra. Body weight on admission, May 14, 52.91 kilos. On May 29, just before yeast preparation was added to diet, 50.51 kilos; on July 31 (time of discharge) 52.32 kilos.

In this case the total daily volume of milk obtained never exceeded 200 c. c.

Case No. 137.—Admitted September 8, 1915; discharged October 20, 1915. White, female, married, has five children, youngest child 5 months old.

Developed pellagrous symptoms in June, 1915, with erythema of hands, vertigo, diarrhœa. On admission she complained of vertigo, burning of tongue, diarrhœa, and epigastric pain. Patient is poorly nourished; has markedly reddened tongue with numerous fissures. Skin over extensor surface of hands reddened, dorsum desquamating; poor memory. Patellar reflexes slightly exaggerated; Romberg negative; hæmoglobin, 78 (Sahli).

Five-months'-old child was nursed at the breast and is still nursing after admission to hospital. Infant is in fair physical condition, although undernourished; does not exhibit any pellagrous symptoms; is fretful and cries a great deal, is put on modified cow's milk, besides being fed at the breast. Infant gradually improved on this treatment; had more color, slept better, and seemed brighter. Patient was on diet A from September 9 to 20, inclusive; thereafter on diet B. Patient left hospital much improved, having gained 2.1 kilos in body weight.

In this case not all of the milk was obtained, as patient's child was left at the breast; therefore no information is available as to the degree of lactation. On September 14, 500 c. c. were obtained, which seems to point to a fairly active lactation.

DISCUSSION OF RESULTS.

Volume of secreted milk.—In this series of cases lactation seemed to be considerably influenced by the severity of the disease, and possibly also by the character of the diet. Cases No. 55 and 86 were of a rather severe type, and were on a diet of low protein content (A). In both cases the total volume of milk was relatively small. It is difficult to decide which one of the above-mentioned factors was most effective in reducing the volume of the milk. We are rather inclined to believe that the disease itself was of greater influence. The total daily volume of milk obtained from each of these two patients never exceeded 200 c. c. On the other hand, lactation seemed to be fairly active in case No. 68 and in case No. 80. Both of these cases represent a milder type of the disease. There is nothing surprising in the reduction of lactation in pellagra when one considers that this disease has a depleting influence on the body and usually results in a marked loss of body weight.

Chemical constituents of milk.—A comparison of the data presented in Tables 18, 19, and 20 seems to justify the following conclusions in regard to the character of the variations of the milk from pellagrins, as compared with normal controls: Lactose, total fat, total nitrogen, and total solids were found to come within normal limits, although all of these constituents were below the average of the normal. The total ash and phosphoric anhydride content were found to be normal. A considerable increase over the normal in the sodium and chlorine was found. The calcium, magnesium, and potassium were all below the normal standard.

As will be seen from Tables 2 and 3, diet A differs considerably from diet B in its content of the various inorganic salts. It is, therefore, rather surprising to find that the inorganic constituents of the milk seem to be very little influenced by the composition of the diet. This observation, however, is in perfect agreement with the results of blood analyses reported by Lewis in this bulletin (p. 41). Here also nothing abnormal could be detected in the salt content of the blood. Hence it is fairly safe to conclude that the mineral metabolism is very little, if at all, disturbed in this disease.

Tables 21 and 22 illustrate the marked influence of the protein intake on the total nitrogen and protein content of the milk. It is seen that an increase of over 100 per cent in the protein intake leads to a considerable increase in the total nitrogen and total protein of the secreted milk. A similar observation is also made in regard to the nonprotein nitrogen. It is quite possible that part of the increase in this latter fraction is due to an increase in antineuritic vitamine, which is probably one of the constituents of this fraction. Diet B, which seems to produce this increase in nonprotein nitrogen, is obviously rich in antineuritic and fat-soluble vitamine, as it contains large amounts of milk, eggs, and meat. Diet A, on the other hand, contains much less of the antineuritic substance.

On the whole, it can be stated definitely that the above-mentioned known constituents of milk do not vary in any marked degree from the normal. The milk of pellagrins may, therefore, be considered as being of fairly normal composition, although some variations from the normal standard are observed.

POSSIBLE EXPLANATIONS OF OCCURRENCE OF PELLAGRA IN BREAST-FED INFANTS.

Several possible explanations for the occurrence of pellagra in breast-fed infants suggest themselves:

First. Pellagra might be regarded as a food intoxication either of exogenous or endogenous origin. (*a*) The food of pellagrous mothers might contain some toxic substances which pass over into the milk and cause an intoxication in the breast-fed infant (exogenous food intoxication): or (*b*) the food of the mother, after digestion, might cause an abnormal metabolism, with the subsequent production within the body of toxic intermediary metabolic substances, which, when secreted by the mammary gland, could give rise to the disease in the infant (endogenous intoxication). On this basis, it would largely depend on the amount of toxic substances secreted with the milk as to whether or not the infant would develop the disease. Obviously, this explanation would hardly account for the large number of breast-fed infants who never develop pellagra, although their mothers may suffer from severe attacks of this disease. Of course, it might be possible that the reduction in the amount of breast milk of such cases would necessitate the feeding of the infant with other food besides breast milk, and in this way the absolute amount of toxic substances consumed by the infant might not be sufficient to cause the intoxication. We observed, however, that pellagrous mothers usually fed their infants very much the same food, besides breast milk, as they themselves consumed. For this reason the addition of this extra food to the breast milk should rather favor the production of the disease in the infant. It should also be emphasized that, up to date, the presence of toxic products in human milk of pellagrins has not been demonstrated. This explanation, therefore, is not very convincing.

Second. The diet of pellagrous mothers might be deficient in certain essential dietary components. Under this condition, the milk secreted by these patients might become deficient in one or several of the normal milk constituents, an interpretation which has much in its favor. Thus it is conceivable that the breast milk might be deficient in proteins containing the necessary amino acids essential for normal growth and maintenance. The results of the milk analyses reported in this paper do not support this view, however, as it was shown that the milk from severe cases of pellagra does not reveal more than a very moderate diminution in the casein and lactalbumen. Both of these proteins are known to contain all the essential amino acids in sufficient amounts for normal growth. The analyses show, furthermore, that the variations in the mineral salts, fat, and lactose of the breast milk are well within the physiological limits. The criticism might be raised that the milk used for the analyses was obtained from pellagrous mothers whose infants did not exhibit unquestionable symptoms of the disease. The writers fully appreciate the justice of this criticism. The clinical material at our disposal did not permit us to analyze the milk of pellagrins whose infants also were affected with the disease. However, if a deficiency of the breast milk in inorganic salts or proteins were the cause of the disease it would be rather surprising not to find definite and pronounced changes in these milk constituents in the samples of milk dealt with in this investigation. It would seem that the severity of the disease in these patients was certainly sufficient to produce such changes in the milk, especially when one considers that the diet of some of these patients was very similar in composition to the diet which the patients consumed previous to their attack of pellagra. As regards the inorganic salts it might be furthermore pointed out that Lewis (p. 42) has found no abnormality in the inorganic salt content of the blood of severe cases of pellagra. Hence it appears very doubtful that a deficiency of the breast milk in adequate protein and inorganic salts may be held responsible for the occurrence of pellagra in breast-fed infants.

There remains still a further explanation, namely, a deficiency of the breast milk in fat-soluble, in antiscorbutic, or in antineuritic vitamine. Normal milk is known to contain an abundance of fat-soluble vitamine and a sufficient amount of antineuritic substance. It is possible that the breast milk of pellagrins may sometimes contain less of these substances than normal milk. If there should exist a deficiency in the breast milk of antineuritic vitamine alone, this milk should produce beriberi and not pellagra. The possibility that a combined deficiency of fat-soluble, antiscorbutic, and antineuritic vitamine may cause the syndrome known as pellagra is not contradicted by our present knowledge of the disease.

The deficiency theory of pellagra would also help to explain the well-established fact that women are very susceptible to this disease during the period of lactation. As far as we are aware, very few, if any, cases have been reported during pregnancy. Is it not possible that during lactation the mother gives up with her milk a relatively large amount of vitamines, more than she can afford to spare for her own metabolism?

The case reported in this paper, of a breast-fed pellagrous infant, whose mother was evidently free from the disease, could also be explained on the basis of the vitamine hypothesis. In this instance, the mother, living on a one-sided diet, may have retained enough vitamines for her own metabolism. Her milk, however, may have been deficient in this respect, with the result that her infant developed the disease.

In those cases where the breast-fed infants of pellagrous mothers do not develop pellagra, it might be argued that the mother provides the infant with a sufficient supply of milk vitamines, to the neglect of her own demands.

In conclusion, it should be pointed out that exactly analogous conditions are met with in a disease which has been definitely accepted as being due to a vitamine deficiency of the diet, namely, beriberi. Here, also, the disease may or may not be transmitted by the mother to the breast-fed infant. Beriberi is one of the most important factors concerned in the high infant mortality in the Philippines. It is very common to find both breast-fed infant and mother affected with beriberi. Hirota (1898) has also reported a few cases of beriberi in breast-fed infants whose mothers did not suffer from this disease. The incidence of beriberi seems also to be rather high in women during lactation. Andrews (1912) has shown by chemical analysis that the human milk in beriberi differs very little in composition from the normal.

Suggestions as to prevention of pellagra in infants.

Finally, a few practical suggestions may not be out of place. It has been our experience that the infants of pellagrous mothers present a poorly nourished and anemic appearance. Very often the mother states that she has not enough milk for her baby, a condition which is not surprising when one takes into consideration the depleted condition of the mother. In many instances we found that the mother had supplemented the breast milk by foods used by the rest of the family, such as biscuits, corn bread, green vegetables, and fat pork. There is no reason why the children of a pellagrin should not grow up in a normal way, provided proper feeding is instituted. A number of such infants were treated at the Pellagra Hospital, by a proper modified cow's milk, and in every case the child thrived and gained in weight. The cotton-mill village population of Spartanburg County has considerable difficulty in procuring high-grade cow's milk; this is especially true during the summer season. Buttermilk is much more easily obtainable and should be recommended, especially for summer feeding of infants. Personal observations by one of the authors (V.) would seem to indicate that it is inadvisable to wean the children of pellagrous mothers, unless absolutely necessary. The best recommendation we can offer is to provide the mother with at least a quart of milk per day, a measure which can be carried out in most cases. The effect of this treatment will often be noticed in both child and mother within two weeks.

Most of these mothers are in urgent need of advice regarding the feeding of children. Here lies a splendid field for divisions of child hygiene of State health departments.

SUMMARY.

A case is described of pellagra in a breast-fed infant, whose mother did not exhibit symptoms of the disease. Suggestions are made as to the proper dieting of pellagrous mothers and their infants.

Chemical analysis of the milk from five cases of uncomplicated pellagra revealed no striking abnormalities, so far as could be determined. The following constituents were found to fall within normal limits, but below the average: Lactose, fat, total nitrogen, and total solids. The total ash and phosphoric anhydride content was normal. The chlorine and sodium were found to be present in larger amounts than normally. The percentage of calcium, magnesium, and potassium was slightly below the normal.

No observations were made on the vitamine content of the milk and it is, therefore, possible that the milk was lacking in some food accessories.

The total volume of the milk was found a great deal diminished in many cases. A change from the low to high protein diet seems to lead to an increase in total and nonprotein nitrogen in the milk.

The findings here reported do not conflict with the vitamine theory of pellagra, although no definite proof of this theory is furnished.

Addendum.

Since this bulletin was submitted for publication an interesting paper by McCollum and Simmonds (Am. J. Physiol., 1916, vol. 45, p. 275) appeared. The reader is referred to this article for an extensive review of the literature on the influence of the diet of the mother on the composition of the secreted milk.

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TABLE 1.—*Food analyses.*

Food.	Moisture.	Ash.	Protein.	Fat.	Carbohydrate.	Calories per gram.		
						Determined small calories.	Calculated small calories.	Physiologic value large calories.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*			
Butter	14.68	1.74	0.87	82.71	....	7,742	....	7.728
Beans [1]	18.27	4.08	22.07	3.01	52.57	3,694	3,693	3.340
Eggs	68.50	1.25	13.44	16.79	....	2,338	2,327	2.112
Cabbage	94.62	.55	.74	.38	3.70	229	229	.217
Ham	52.95	8.07	24.07	14.94	....	2,745	2,761	2.376
Corn sirup	27.56	1.40	....	....	71.04	2,806	....	2.806
Meat	75.40	2.17	21.12	1,27	....	1,323	1,322	.984
Milk	86.37	.78	3.58	4.78	4.49	833	833	.775
Orange juice	92.13	....	....	....	7.87	....	323	.323
Potatoes:								
Sweet	71.46	1.10	1.24	.15	26.05	1,152	1,153	1.133
Irish	83.30	.77	1.58	.01	14.35	677	679	.654
Prunes	26.25	2.17	2.01	.53	69.04	3,004	2,992	2.060
Pork fat	3.62	2.71	3.09	90.58	....	8,600	....	8.551
Rice [1]	16.38	.45	7.41	.06	75.70	3,527	3,532	3.413
Sugar	.14	.12	....	....	99.74	3,939	....	3.939
Turnips	95.91	.35	.21	.05	3.48	157	159	.156
Turnip tops	90.15	1.07	2.52	.53	5.72	427	426	.385
Wheat flour [1]	19.26	.41	8.54	1.23	70.56	3,505	3,494	3.357
Wheat bread	38.58	1.44	8.33	3.40	48.24	2,769	2,768	2.635
Corn meal [1]	14.07	1.27	7.33	3.22	74.11	3,776	3,756	3.639
Do [1]	16.48	1.51	9.92	1.70	70.40	3,610	3,610	3.451
Do. [1, 2]	30.67	1.35	8.06	3.87	56.06	3,120	3,117	2.988
Do	11.12	1.52	7.24	5.72	74.40	4,006	3,995	3.879
Average	13.89	1.43	8.16	3.55	72.97	3,797	3,787	3.656
Hominy	13.28	.51	7.72	2.45	76.04	3.783	3,786	3.662
Do. [1]	17.11	.69	8.13	1.54	72.53	3,577	3,580	3.450
Do. [1]	20.52	.33	8.19	.12	70.84	3,476	3,483	3.352
Average	16.97	.51	8.01	1.37	73.14	3,612	3.616	3.488

[1] Samples purchased at Saxon Mill Store.

[2] Excluded from average because of high moisture content.

TABLE 2.—*Composition of diet A, complete.*

Food.	Amount of food (gms.).	Protein (gms.).	Fat (gms.).	Carbohydrate (gms.).	Calories.	CaO (gms.).	MgO (gms.).	Na_2O (gms.).	K_2O (gms.).	Cl (gms.).	P_2O_5 (gms.).
Wheat bread	300	25.0	10.2	144.7	790	0.075	0.081	0.120	0.438	0.210	0.600
Butter	30	.3	24.8	....	232	.006	.001	....	.006	....	.009
Cabbage	100	.7	.4	3.7	22	.068	.026	.050	.450	.030	.090
Corn meal	50	4.1	1.8	36.5	183	.007	.065	.015	.085	....	.150
Ham	25	6.0	3.7	....	59	.008	.010	....	....	....	....
Hominy	75	6.0	1.0	54.9	262	.010	.097	.022	.128	....	.225
Corn sirup	30	....	....	21.3	84	....	....	....	....	....	....
Pork	50	1.5	45.3	....	428	....	....	....	....	....	....
Potatoes	150	2.4	....	21.5	98	.024	.054	.037	.800	.045	.210
Prunes	30	.6	.2	20.8	88	.018	.024	.030	.360	.003	.075
Turnip tops	100	.5	.5	5.7	38	.480	.050	.110	.370	.170	.110
Sugar	40	....	....	39.9	158	....	....	....	....	....	....
Milk	40	1.4	1.9	1.8	31	.068	.008	.027	.068	.048	.086
Total		50.5	89.8	350.8	2,473	.764	.416	.713	3.146	.506	2.110

NOTE.—The salt content was calculated from the analyses by Sherman.

TABLE 3.—*Composition of diet B, complete.*

Food.	Amount of food (gms.).	Protein (gms.).	Fat (gms.).	Carbohydrate (gms.).	Calories.	CaO (gms.).	MgO (gms.).	Na_2O (gms.).	K_2O (gms.).	Cl (gms.).	P_2O_5 (gms.).
Wheat bread	300	25.0	10.2	144.7	790	0.075	0.081	0.120	0.438	0.210	0.600
Butter	45	.5	37.2	....	348	.009	.001	....	.009	....	.014
Corn meal	50	4.1	1.8	36.5	183	.007	.065	.015	.085	....	.150
Eggs	100	13.4	16.8	....	211	.093	.015	.200	.165	.100	.370
Meat	100	21.1	1.3	....	98	.011	.040	.090	.420	.050	.500
Orange juice	100	....	....	7.9	32	.050	.020	.010	.220	.010	.030
Potatoes	150	2.4	....	21.5	98	.024	.054	.037	.800	.045	.210
Prunes	30	.6	.2	20.8	88	.018	.024	.030	.360	.003	.075
Sugar	40	....	....	39.9	158	....	....	....	....	....	....
Milk	1,000	35.8	47.8	44.9	775	1.680	.190	.680	1.710	1.200	2.150
Total		102.9	115.3	316.2	2,781	1.967	.490	1.182	4.207	1.618	4.099

NOTE.—The salt content was calculated from the analyses by Sherman.

TABLE 4.—*Case No. 55. Food intake (in grams).*

DIET A.

	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy.[1]	Corn sirup.	Pork.	Potatoes.	Turnip tops.	Sugar.	Milk.	Coffee.	Salt.	Vitamine.	Prunes.
Mar. 5	276	....	100	50	25	42	30	19	150	64	....	....		1		30
6	300	15	100	50	25	9	30	31	....	100	....			2		30
7	295	....	87	50	25	....	30	32	74	100	....			3		30
8	300	15	79	50	25	....	30	41	....	100	....	....		1		....
9	241	....	12	50	25	....	25	38	150	100	....	....		2		....
10	290	....	18	50	25	....	30	37	9	100	....	....		....	40	....
11	290	....	53	50	25	10	30	32	....	100	....				40	....
12	296	....	76	50	25	....	30	42	....	100	....			....	40	4
13	299	....	14	50	25	....	30	39	106	98	....			1	40	....
14	300	....	100	50	25	6	30	47	150	100	....			3	40	....
15	300	15	100	50	25	40	25	28	22	100	....			5	40	....
16	300	15	50	50	25	50	30	48	150	85	....			3	40	....
17	295	14	40	50	25	69	24	40	137	95	....			8	40	....
18	300	30	100	50	25	57	30	46	52	100	....	....		5	40	30
19	298	30	100	42	25	38	30	39	150	100	....			6	40	3
20	293	16	100	50	25	60	30	25	41	100	....	....		6	40	30
21	300	....	100	50	25	68	25	41	150	100	....			7	40	30
22	298	11	100	50	25	72	30	41	150	100	....			4	40	30
23	300	30	100	50	25	75	30	34	150	100	....			7	40	30
24	300	30	100	50	25	75	30	43	150	100	....			8	30	30
25	255	30	100	50	25	74	30	36	....	100				9	30	30
Average	287	12	78	50	25	35	29	37	85	98	....		....	4	30	15

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[1] Cubic centimeters.

TABLE 5.—*Summary of Table 4.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
	Grams.	*Grams.*	*Grams.*	*Grams.*
Wheat bread	756	24.0	9.8	138.4
Butter	93	.1	9.9	....
Cabbage	17	.6	.3	2.9
Corn meal	183	4.1	1.8	36.5
Ham	59	6.0	3.7	....
Hominy	122	2.8	.5	25.6
Corn sirup	81	....	....	20.6
Pork	316	1.1	33.5	....
Potatoes	56	1.3	....	12.2
Prunes	44	.3	.1	10.4
Turnip tops	38	2.5	.5	5.6
Total	1,765	42.8	60.1	252.2

TABLE 6.—*Case No. 68. Food intake (in grams).*

DIET A.

	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy.	Corn sirup.	Pork.	Potatoes.	Prunes.	Turnip tops.	Sugar.	Milk.	Coffee.	Salt.
Mar. 26	290	25	100	50	25	56	30	43	135	30	95	40	40	350	7
27	196	30	100	50	25	70	30	43	150	30	100	40	40	350	7
28	300	30	100	50	25	75	30	41	150	30	100	40	40	350	8
29	300	30	100	50	25	75	30	43	150	30	100	40	40	350	11
30	300	30	100	50	25	75	30	44	150	30	100	40	40	350	12
31	300	30	100	50	25	75	30	43	150	30	100	40	40	350	12
Apr. 1	300	30	100	50	25	75	30	43	116	30	100	40	40	350	13
2	300	30	100	50	25	75	30	42	150	30	100	40	40	350	16
3	300	45	103	50	25	70	30	28	150	30	104	40	40	325	10
4	300	30	100	50	25	73	30	41	150	30	101	40	40	350	10
5	300	30	104	50	25	76	30	46	150	30	105	40	40	350	2
6	300	18	95	50	25	75	30	47	126	30	95	40	40	350	12
7	300	30	98	50	25	66	30	48	150	30	99	40	40	350	14
8	300	30	104	49	25	69	30	45	150	30	109	40	40	350	8
9	300	30	103	50	25	75	30	49	150	30	107	40	40	350	13
10	300	30	99	50	25	78	30	47	150	30	99	40	40	350	10
11	300	30	99	50	25	75	30	48	150	30	100	40	40	300	21
12	300	30	98	54	25	67	30	44	150	30	44	40	40	350	21
13	300	30	102	50	25	82	30	46	150	30	100	40	40	350	15
14	300	30	99	50	25	75	30	46	150	30	97	40	40	350	1
15	300	30	99	50	25	82	30	46	150	30	91	40	40	350	22
16	300	30	106	50	25	75	30	65	150	30	100	40	40	350	19
17	300	30	94	50	25	65	30	46	150	30	99	40	40	350	22
18	300	30	109	50	25	75	30	38	150	30	95	40	40	350	12
19	300	30	99	50	25	75	30	47	150	30	100	40	40	350	18
20	300	30	99	50	25	75	30	49	150	30	99	40	40	350	7
21	300	30	99	50	25	69	30	45	150	30	99	40	40	350	23
22	300	30	99	50	25	75	30	46	150	30	100	40	40	350	21
23	300	30	100	50	25	75	30	46	150	27	101	40	40	350	23
24	300	30	100	50	25	73	30	47	150	30	98	40	40	350	27
25[1]	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	296	30	100	50	25	73	30	45	148	30	98	40	40	349	14

[1] Dismissed.

TABLE 7.—*Summary of Table 6.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
		Grams.	*Grams.*	*Grams.*
Wheat bread	780	24.7	10.1	142.8
Butter	232	0.3	24.8	......
Cabbage	22	0.7	0.4	3.7
Corn meal	183	4.1	1.8	36.5
Ham	59	6.0	3.7	......
Hominy	255	5.8	1.0	53.4
Corn sirup	84	......	......	21.3
Pork	385	1.4	40.8	......
Potatoes	97	2.3	......	21.2
Prunes	88	0.6	0.2	20.8
Turnip tops	38	2.5	0.5	5.6
Sugar	158	......	......	39.9
Milk	31	1.4	1.9	1.8
Average in food	2,412	49.8	85.2	347.0
Average in human milk	365	5.5	18.3	42.1

TABLE 8.—*Case No. 80. Food intake (in grams).*

DIET A.

	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy.	Corn sirup.	Pork.	Potatoes.	Prunes.	Turnip tops.	Sugar.	Milk.	Coffee.	Salt.
Apr. 25	216	30	100	22	25	11	30	39	44	24	67	40	40	350	2
26	202	30	100	16	25	20	30	40	150	30	70	40	40	350	5
27	142	30	100	24	25	26	30	34	150	30	24	40	40	350	2
28	192	30	......	50	25	24	30	7	......	30	......	40	40	350	1
29	223	30	100	24	25	23	30	38	150	30	100	40	40	350	3
30	234	15	100	28	18	15	30	43	150	30	100	40	40	350	5
May 1	275	30	100	27	25	31	30	42	150	30	100	40	40	350	5
2	232	30	99	50	25	31	30	45	150	30	100	40	40	350	4
3	200	30	99	39	25	26	30	44	150	30	100	40	40	350	7
4	232	30	100	38	25	12	30	39	150	30	85	40	40	350	5
6	190	30	100	40	25	16	30	45	150	33	100	40	40	350	5
5	113	30	100	50	25	18	30	36	150	33	100	40	40	350	6
7	241	30	100	35	25	16	30	42	150	30	100	40	40	350	2
8	273	30	89	30	25	15	30	40	150	30	91	40	40	350	9
9	280	30	98	25	25	19	30	39	150	30	95	40	40	350	6
Average	216	29	92	33	25	20	30	38	133	30	82	40	40	350	4

TABLE 9.—*Summary of Table 8.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
		Grams.	*Grams.*	*Grams.*
Wheat bread	569	18.0	7.3	104.3
Butter	224	.3	24.0	......
Cabbage	20	.7	.3	3.4
Corn meal	122	2.7	1.2	24.3
Ham	59	6.0	3.7	......
Hominy	71	1.6	.3	14.9
Corn sirup	84	......	......	21.3
Pork	325	1.2	34.4	......
Potatoes	87	2.1	......	19.1
Prunes	88	.6	.2	20.8
Turnip tops	32	2.1	.4	4.7
Sugar	158	......	......	39.9
Milk	31	1.4	1.9	1.8
Average in food	1,870	36.7	73.7	245.5
Average in human milk	148	2.1	6.2	19.9

TABLE 10.—*Case No. 80. Food intake (in grams).*

DIET B.

	Wheat bread.	Butter.	Corn meal.	Eggs.	Meat.	Orange juice.	Potatoes.	Prunes.	Sugar.	Milk.	Coffee.	Salt.
May 10	256	45	34	100	100	100	150	33	40	1,200	350	5
11	226	45	23	100	79	100	150	30	40	1,000	350	3
12	246	45	16	100	46	100	150	30	40	1,000	350	5
13	225	45	17	100	......	100	150	30	40	1,000	350	4
14	240	35	50	100	100	100	43	30	40	1,000	350	6
15	251	45	50	100	......	60	150	30	40	1,000	350	5
16	285	45	19	100	40	100	150	30	40	1,000	350	1
17	255	45	18	100	59	100	150	30	40	1,000	350	4
18	230	45	10	100	34	100	150	30	40	1,000	350	5
19	258	45	17	100	66	100	150	6	40	1,000	350	5
20	201	37	18	100	43	100	115	30	40	1,000	350	3
21	237	45	50	100	48	100	150	30	40	1,000	350	3
22	260	45	76	100	68	100	103	30	40	1,000	350	4
23	249	45	38	100	100	100	106	30	40	1,000	350	?
24	250	45	55	100	26	100	150	30	40	1,000	350	?
25	237	45	50	100	17	100	150	30	40	1,000	350	?
Average	244	44	34	100	52	98	136	29	40	1,012	350	4

TABLE 11.—*Summary of Table 10.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
		Grams.	*Grams.*	*Grams.*
Wheat bread	643	20.3	8.3	117.6
Butter	340	.4	36.4	....
Corn meal	125	2.8	1.2	24.5
Eggs	211	13.4	16.8	....
Meat	51	10.6	.7	....
Orange juice	32	....	....	7.8
Potatoes	89	2.1	....	19.5
Prunes	85	.6	.2	20.1
Sugar	158	....	....	39.9
Milk	785	36.2	48.4	45.5
Average in food	2,519	86.4	112.0	274.9
Average in human milk	52	.8	2.2	7.0

TABLE 12.—*Case No. 86. Food intake (in grams).*

DIET A.

	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy.	Corn sirup.	Pork.	Potatoes.	Prunes.	Turnip tops.	Sugar.	Milk.	Coffee.	Salt.	Vitamine.
May 15	131	18	75	....	8	75	10	26	125	30	18	40	40	350	14	....
16	116	24	....	....	25	25	....	....	....	....	....	40	40	350	10	....
17	154	30	72	....	25	75	....	35	102	15	....	40	40	350	10	....
18	229	30	....	....	11	75	30	21	99	15	10	40	40	350	7	....
19	136	30	80	35	11	66	....	44	31	20	100	40	40	350	5	....
20	148	30	....	....	25	70	....	19	59	....	46	40	40	350	9	....
21	104	30	....	....	25	58	30	....	....	19	....	40	40	350	6	....
22	126	30	99	....	25	37	....	27	....	....	....	40	40	350	6	....
23	87	23	32	27	25	23	....	30	33	....	56	40	40	350	....	....
24	184	30	....	....	25	24	....	24	102	....	....	20	20	175	....	....
25	100	30	14	....	25	8	....	18	....	....	....	20	20	175	....	....
June 4	168	30	100	....	25	....	30	37	....	30	100	40	40	350	....	....
5	174	30	100	....	25	33	30	23	150	30	....	40	40	350	....	....
6	173	30	90	19	25	46	30	35	82	32	42	40	40	350	....	[1] 35
Average	145	28	47	6	22	44	11	24	56	14	27	37	37	325	8	....

[1] Cubic centimeters.

TABLE 13.—*Summary of Table 12.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
		Grams.	*Grams.*	*Grams.*
Wheat bread	383	12.0	4.4	69.2
Butter	216	0.2	23.2	....
Cabbage	10	0.3	0.2	1.7
Corn meal	22	0.4	0.2	[illegible].4
Ham	52	5.3	3.3	....
Hominy	153	3.5	0.6	32.2
Corn sirup	31	....	....	7.8
Pork	205	0.7	21.7	....
Potatoes	37	0.9	....	8.0
Prunes	41	0.3	0.1	9.7
Turnip tops	10	0.7	0.1	1.5
Sugar	146	....	....	36.9
Milk	29	1.3	1.8	1.7
Total	1,335	25.6	55.6	173.1

TABLE 14.—*Case No. 137. Food intake (in grams).*

DIET A.

	Wheat bread.	Butter.	Cabbage.	Corn meal.	Ham.	Hominy.	Corn sirup.	Pork.	Potatoes.	Prunes.	Sugar.	Milk.	Coffee.
Sept. 13	262	30	46	50	25	64	30	26	113	30	40	40	350
14	287	30	47	50	25	68	30	15	142	30	40	40	350
15	235	30	37	49	25	60	30	33	107	30	40	40	350
16	285	30	......	50	25	35	30	22	80	30	40	40	350
17	274	30	40	50	18	64	30	26	56	30	40	40	350
18	292	30	......	50	25	75	30	31	56	30	40	40	350
19	274	30	28	50	25	52	30	23	103	30	40	40	350
20	231	30	......	50	25	68	30	26	150	30	40	40	350
Average	268	30	25	50	24	61	30	25	101	30	40	40	350

TABLE 15.—*Summary of Table 14.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
		Grams.	*Grams.*	*Grams.*
White bread	706	22.3	9.1	129.2
Butter	232	.3	24.8	....
Cabbage	5	.2	.1	.9
Corn meal	183	4.1	1.8	36.5
Ham	57	5.8	3.6	....
Hominy	213	4.9	.8	44.7
Corn sirup	84	....	....	21.3
Pork	214	.8	22.6	....
Potatoes	66	1.6	....	14.4
Prunes	88	.6	.2	20.8
Sugar	158	....	....	39.9
Milk	31	1.4	1.9	1.8
Average in food	2,037	42.0	64.9	309.5
Average in human milk	72	1.2	3.7	8.0

TABLE 16.—*Case No. 137. Food intake (in grams).*

DIET B.

	Wheat bread.	Butter.	Corn meal.	Eggs.	Meat.	Orange juice.	Potatoes.	Prunes.	Sugar.	Milk.	Coffee.
Sept. 28	300	45	50	100	100	100	30	30	40	1,000	350
29	300	45	50	100	100	100	48	30	40	1,000	350
30	300	45	50	100	100	100	24	30	40	1,000	350
Oct. 1	300	45	50	100	100	100	38	30	40	1,000	350
2	300	45	50	100	100	100	93	30	40	1,000	350
3	300	45	50	100	100	100	45	30	40	1,000	350
4	300	45	45	100	100	100	85	30	40	1,000	350
5	300	45	50	21	71	100	125	30	40	1,000	350
6	300	45	50	76	100	100	92	30	40	1,000	350
7	300	45	50	100	100	100	150	30	40	1,000	350
8	300	45	50	100	100	100	150	30	40	1,000	350
9	300	45	50	100	100	100	150	30	40	1,000	350
10	300	45	50	78	100	100	150	30	40	1,000	350
11	300	45	50	100	100	100	150	30	40	1,000	350
Average	300	45	50	91	98	100	95	30	40	1,000	350

TABLE 17.—*Summary of Table 16.*

Food.	Calories.	Protein.	Fat.	Carbohydrate.
Wheat bread	791	25.0	10.2	144.7
Butter	348	.5	37.2	....
Corn meal	183	4.1	1.8	36.5
Eggs	192	12.2	15.3	....
Meat	96	20.7	1.2	....
Orange juice	32	....	....	7.9
Potatoes	62	1.5	....	13.6
Prunes	88	.6	.2	20.8
Sugar	158	....	....	39.9
Milk	775	35.8	47.8	44.9
Average in food	2,725	100.4	113.7	308.3
Average in human milk	90	1.8	4.2	10.6

TABLE 18.—*Chemical composition of human milk in pellagra.*

Milk sample No.	Case No.	Age of mother (years).	Age of child (months).	No. of child.	Amount of milk (c. c.).	Lactose (per cent).	Fat (per cent).	Total nitrogen (per cent).	Total solids (per cent).	Total ash (per cent).	Casein nitrogen (per cent).	Albumin nitrogen (per cent).	Body weight (kilos).
1	137	33	5	5	493	6.47	2.32	0.145	11.65	0.214	0.092	0.024	.....
2	....	....	....	....	368	6.47	3.66	.149	13.10	.205	....	....	32.53
3[1]	....	....	....	....	451	6.28	2.82	.192	12.78	.222	.112	.037	34.01
4[1]	....	....	....	....	552	6.40	2.79	.183	12.35	.215	.106	.034	......
5[1]	....	....	....	....	561	6.80	2.32	.175	11.54	.225	.093	.048	......
6[1]	....	....	....	....	500	6.80	2.58	.179	12.23	.225	.104	.037	34.62
7	86	26	5½	4	645	6.67	2.34	.161	11.52	.229	.089	.037	52.91
8	....	....	....	....	450	6.80	2.66	.210	12.57	.251	.159	.033	51.71
9	55	28	7	3	365	6.73	2.95	.176	12.16	.238	.097	.040	41.13
10	68	32	10	5	540	7.16	2.34	.138	11.33	....	....	....	38.15
11	....	....	....	....	610	6.60	2.17	.142	11.88	....	....	....	38.00
12	....	....	....	....	640	7.08	3.51	.146	12.02	....	....	....	......
13	....	....	....	....	680	6.94	2.76	.148	11.95	....	....	....	38.32
14	....	....	....	....	659	7.31	2.51	.145	11.92	....	....	....	......
15	....	....	....	....	598	7.16	2.77	.140	11.96	....	....	....	......
16	....	....	....	....	591	7.01	3.28	.138	12.09	....	....	....	39.63
17	....	....	....	....	513	6.94	3.36	.141	12.49	....	....	....	......
18	....	....	....	....	538	6.73	3.02	.141	12.44	....	....	....	......
19	....	....	....	....	499	6.73	3.13	.141	12.14	....	....	....	......
20	....	....	....	....	447	6.67	3.06	.140	12.08	....	....	....	......
21	....	....	....	....	434	6.67	3.16	.137	12.64	....	....	....	39.52
22	....	....	....	....	422	6.67	3.02	.138	12.48	....	....	....	......
23	80	26	10	5	130	7.08	2.58	.126	11.21	....	....	....	35.02
24	....	....	....	....	354	7.08	1.64	.104	10.68	....	....	....	......
25	....	....	....	....	279	7.01	2.32	.111	11.18	....	....	....	......
26	....	....	....	....	374	6.94	2.13	.122	11.04	....	....	....	35.21

---

[1] All milk samples, with the exception of samples Nos. 3 to 6, inclusive, were obtained while mothers were on low protein diets (35-45 grams protein daily). Samples Nos. 3 to 6 were obtained while the mother was on a diet containing 100 grams protein daily.

TABLE 19.—*Chemical composition of human milk in pellagra: mineral salt content.*

No.	Case No.	Age of mother (years).	Age of child (months).	No. of child.	Amount of milk (c. c.).	Total ash.		Calcium oxide.		Magnesium oxide.		Potassium oxide.		Sodium oxide.		Chlorine.		Phosphorus pentoxide.		Body weight (kilos).
						Determined (per cent).	Calculated (per cent).	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	
1	137	33	5	5	493	0.214	0.2134	0.0270	13	0.0028	1.3	0.0492	23	0.0262	12	0.0621	29	0.0161	22	......
2	....	....	....	....	368	.205	.1972	.0311	16	.0033	1.7	.0446	23	.0110	6	.0568	29	.0504	26	32.53
3	....	....	....	....	451	.222	.2397	.0332	14	.0050	2.1	.0525	22	.0411	17	.0533	22	.0546	23	34.01
4	....	....	....	....	552	.215	.2215	.0350	16	.0050	2.3	.0510	23	.0226	10	.0542	21	.0537	24	......
5	....	....	....	....	561	.225	.2251	.0340	15	.0047	2.1	.0518	23	.0284	13	.0533	24	.0529	23	......
6	....	....	....	....	500	.225	.2287	.0314	14	.0040	1.8	.0545	24	.0322	14	.0551	24	.0515	23	34.62
7	86	26	5½	4	645	.229	.2618	.0313	12	.0076	2.9	.0624	24	.0448	17	.0835	32	.0322	12	52.91
8	....	....	....	....	450	.251	.2756	.0318	12	.0081	2.9	.0600	22	.0595	22	.0852	31	.0310	11	51.71
9	55	28	7	3	365	.238	.2350	.0233	10	.0073	3.1	.0480	20	.0324	14	.0914	39	.0326	14	41.13
10	68	32	10	5	771	.192	.1878	.0272	14	.0032	1.7	.0378	20	.0244	13	.0586	31	.0366	20	38.15
11	....	....	....	....	564	.190	.1849	.0289	16	.0039	2.1	.0361	20	.0240	13	.0568	31	.0352	19	...
12	....	....	....	....	600	.189	.1855	.0286	15	.0040	2.2	.0383	21	.0242	13	.0551	30	.0353	19	......
13	....	....	....	....	610	.183	......	.0273	...	.0042	...	......	...	......	...	.0568	...	.0363	...	38.00
14	....	....	....	....	646	.210	.2111	.0284	13	.0036	1.7	.0591	28	.0228	11	.0639	30	.0333	16	......
15	....	....	....	....	626	.200	.1978	.0300	15	.0043	2.2	.0358	18	.0336	17	.0595	30	.0346	18	39.63
16	....	....	....	....	554	.216	.2160	.0229	14	.0040	1.9	.0504	23	.0360	17	.0621	29	.0336	16	......
17	80	26	10	5	....	.205	.2039	.0319	16	.0060	2.9	.0372	18	.0215	11	.9745	37	.0328	16	35.02
18	....	....	....	....	....	.....	.2532	.0362	14	.0079	3.1	.0460	18	.0330	13	.0958	38	.0353	14	36.01
19	....	....	....	....	....	.....	.2857	.0374	13	.0071	2.5	.0545	19	.0530	19	.0958	34	.0379	13	36.51
Average							.2236	.0309	14	.0051	2.3	.0483	22	.0317	14	.0676	30	.0399	18	......
Maximum							.2857	.0374	16	.0081	3.1	.0624	28	.0595	22	.0958	39	.0546	26	......
Minimum							.1849	.0233	10	.0028	1.3	.0358	18	.0110	6	.0533	22	.0310	11	......

TABLE 20.—*Analyses of human milk by Holt, Courtney, and Fales.*[1]

	Calcium oxide.		Magnesium oxide.		Potassium oxide.		Sodium oxide.		Chlorine.		Phosphorus pentoxide.	
	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.
Maximum	0.0545	25	0.0097	4.9	0.0688	34	0.0204	10	0.0444	21	0.0514	21
Minimum	.0354	20	.0067	3.2	.0531	27	.0031	2	.0304	14	.0212	13
Average	.0458	22	.0074	3.9	.0609	30	.0132	6	.0358	17	.0345	17

TABLE 21.—*Average analyses of human milk by other investigators.*

	Calcium oxide.		Magnesium oxide.		Potassium oxide.		Sodium oxide.		Chlorine.		Phosphorus pentoxide.	
	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.	Per cent of milk.	Per cent of ash.
Maximum	0.0567	25	0.0116	5.4	0.0848	46	0.0258	16	0.0468	24	0.0585	23
Minimum	.0328	13	.0060	2.1	.0522	25	.0049	2	.0244	10	.0260	12
Average	.0416	20	.0082	3.7	.0632	31	.0189	10	.0340	18	.0409	18

TABLE 22.— *Case No. 137. Effect of food on total nitrogen in milk.*

[Age, 33 years; 5 months post partum.]

Date.	Average daily protein intake.	In the milk.				
		Total nitrogen.	Protein nitrogen.	Per cent of total nitrogen.	Non-protein nitrogen.	Per cent of total nitrogen.
	Grams.	*Per cent.*	*Per cent.*		*Per cent.*	
Sept. 14–17	42.0	0.145	0.115	79	0.026	18
17–21	42.0	.149	.118	79	.031	21
Sept. 29–Oct. 2	100.4	.192	.148	77	.041	21
Oct. 2–5	100.4	.183	.140	76	.038	21
5–8	100.4	.175	.141	81	.038	22
8–12	100.4	.179	.145	81	.037	21

TABLE 23.—*Case No. 80. Effect of food on total nitrogen in milk.*

[Age, 26 years; 10 months post partum.]

Date.	Average daily protein intake.	Total nitrogen in the milk.
	Grams.	*Per cent.*
April 26–27	36.7	0.126
29–30	36.7	.104
May 1–2	36.7	.111
8–9	36.7	.122
16–17	86.4	.140
20–21	86.4	.172
21–22	86.4	.172
23–24	86.4	.210
24–25	86.4	.206

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[1] From Holt, Courtney, and Fales. American Journal Diseases of Children. Vol. 10, No. 4.

TABLE 24.—*Composition of human milk in beriberi.*

[From Andrews.]

Case No.	Volume.	Specific gravity.	Water (per cent).	Fat (per cent).	Sugar (per cent).	Protein (per cent).	Ash (per cent.)	Calcium oxide CaO parts per 1,000 of ash.	Phosphorus pentoxide P_2O_5 parts per 1,000 of ash.
1[1]		1.028	87.30	4.00	7.00	1.50	0.20	0.328	0.473
2		1.032	88.26	2.82	7.70	.92	.30	1.603	.946
2	215	1,032	88.95	2.29	7.16	1.50	.10		
3		1.032	89.75	1.76	7.53	.85	.11		
4	255	1,041	85.75	4.05	8.36	1.50	.34		
4	195	1,029	88.27	3.52	5.22	2.70	.29		
5	88	1.029	90.37	1.76	6.51	1.30	.06		
6	120	1.036	89.43	1.06	7.71	1.70	.10	1.072	.323
7	46	1.020	90.52	3.52	4.98	.90	.08		
8	130	1.033	89.03	2.11	7.61	1.15	.10	.742	.471
8	107	1.034	88.39	2.46	7.44	1.50	.21	1.312	.626
9	149	1.033	90.14	1.06	7.64	.90	.26	1.502	.663
10		1.038	88.10	1.76	8.54	1.50	.10		
11	51	1.029	88.27	3.52	6.66	1.40	.15		
12		1.034	87.12	3.52	7.60	1.50	.26		

[1] The first line of figures in the table shows the percentage of the various constituents of milk of the average normal woman as given by Holt. The calcium oxide and phosphorus pentoxide, however, are taken from Hammerstein's Physiological Chemistry.

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HYGIENIC LABORATORY BULLETIN No. 117

JUNE, 1919

FILIARIASIS IN SOUTHERN UNITED STATES

By

EDWARD FRANCIS
SURGEON, U. S. PUBLIC HEALTH SERVICE

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FILARIASIS IN SOUTHERN UNITED STATES.

By Edward Francis,
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INTRODUCTION.[1]

For the past 30 years filariasis has been known to be endemic at Charleston, S. C., and inasmuch as Charleston's climatic conditions and the species of mosquitoes found there are duplicated in the coast cities of the South, the inference has become general that filariasis is a widespread infection of our southern cities.

The United States Public Health Service, in taking cognizance of the extreme prevalence of filarial infection at Charleston, has conducted a series of investigations having the following objects in view:

(*a*) A filarial survey of a number of southern cities for the purpose of determining, by blood examination, the extent and degree of prevalence of the infection in their population.

(*b*) The determination of the species of mosquito which was acting as the mosquito host and carrier of the infection at Charleston.

(*c*) A determination of the best means of eradicating or preventing the spread of the infection.

FILARIASIS DEFINED.

Filariasis of man is a condition characterized by the presence in the blood of microscopic, slender, snakelike, motile microfilariæ (round worms) which have a length about 30 times the diameter of a red blood corpuscle and a width about equal to the diameter of a red blood cell; they have a rounded head and a tapering tail; they are the offspring of adult female parent worms permanently located in some tissue of the body and discharging their young into the circulation. The parent worms in the case of *Filaria bancrofti* are located in the lymphatics and lymph glands of man; the parent worms of *Filaria loa*, *Filaria perstans*, and *Filaria demarquai* are located in the connective tissue of man. *Filaria bancrofti* is by far the most widespread of the human filariæ and is the species of which this bulletin treats, and for that reason a short general description of that species will now be given, together with some of the symptoms which it causes. *Filaria bancrofti* is found in practically all tropical countries and one endemic focus has been located in the United States—at Charleston, S. C. As the blood of man is the normal habitat of this microfilaria it was at first called *Filaria sanguinis hominis.*

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[1] Manuscript submitted for publication Nov. 5, 1918.

A most interesting phenomenon connected with this microfilaria is its night activity and for that reason it has also been called *Filaria nocturna;* the blood of a filarial patient examined during the hours around midnight may show microfilariæ in great numbers, whereas the blood from the same patient examined in the hours around midday may show only one or two parasites. An estimated number of 40 or 50 millions of microfilariæ circulating in the blood may give rise to no symptoms since their size permits them to traverse the capillaries unobstructed. "The healthy, fully formed microfilariæ have, so far as we can tell, no pathogenic properties whatever; the parent worm and the immature products of conception alone are dangerous." (Manson.)

The adult worms may obstruct the thoracic duct or occlude the smaller lymphatic vessels or the lymph glands, resulting in a damming back of the lymph, giving rise to lymph œdema of the undrained area, and due to this increased lymph pressure there results a condition of lymphatic varix or a varicose dilated state of the lymphatic vessels which causes enlargement of the part affected; hence "lymph scrotum," "varicose groin glands" and "varicose axillary glands." If the dilated lymphatic vessels located in the tissues surrounding the kidneys, bladder, testicle or peritoneum become overdistended and rupture, there results a chyluria, chylocele or chylous ascites.

Elephantiasis, although common where *Filaria bancrofti* abounds, is not usually accompanied by microfilariæ in the blood; there are, however, reasons for believing that elephantiasis is a filarial disease in which the female parent worm located in a lymphatic vessel or lymph gland of the effected part has aborted her immature ova, which, on account of having a larger diameter than that of the healthy fully formed microfilariæ cause embolism of the lymphatic glands, resulting in great enlargement or elephantiasis of the leg, arm, breast, scrotum or vulva.

Filariasis is a mosquito-borne disease and several species of mosquito have been found capable of conveying the infection. Some of the known efficient hosts of *Filaria bancrofti* are *Culex fatigans, Stegomyia pseudoscuttelaris*, and *Anopheles maculipennis.*

THE LIFE CYCLE OF FILARIA BANCROFTI.

The adult filariæ are males and females and are found only in the lymphatic system of man. The approximate length of the males is 1½ inches and that of the females is 3½ inches. The number of adult filariæ found in a single individual is small; eight worms would be a large number for one person. Conjugation of the sexes is essential to multiplication and takes place only within the lymphatic system. Multiplication is brought about by the expulsion of embryos by a gravid female. The number of embryos which one female may discharge is very large—probably at least 1,000. The embryos escape from the lymphatic system and circulate in the patient's blood as microfilariæ.

The microfilariæ do not multiply in the blood; any increase in the number of microfilariæ in the blood comes from additional ones thrown into the blood stream from a parent worm located in a lymph gland. The microfilariæ live for a long time in the blood; probably for years. They circulate awaiting the opportunity of being imbibed by a biting mosquito. The mosquito in drawing blood into its stomach draws in also the microfilariæ which after loosing their sheaths in the mosquito's stomach, pierce the stomach wall and pass to the thoracic muscles where they undergo metamorphosis. After a variable time of approximately two weeks spent in the thoracic muscles, the larvæ migrate to the proboscis and there await an opportunity to get back into man, which opportunity presents itself when the mosquito again bites an individual.

During their stay in the mosquito no multiplication of larvæ takes place. The larvæ when they are ready to leave the proboscis measure one-twenty-fifth to one-sixteenth inch in length.

The larvæ on being delivered back to man by the bite of an infected mosquito pass by way of the lymphatics to the nearest lymph gland where they grow to be adult filariæ.

The cycle, as outlined, starts with the parent worm located in a lymphatic gland giving off her microscopic embryos and ends with those embryos developed into 3-inch worms and again located in a lymphatic gland.

I. FILARIAL SURVEY OF TEN SOUTHERN CITIES.

Charleston, S. C.

John Guiteras,[1] in 1886, then a passed assistant surgeon in the United States Public Health Service, stationed at Charleston, S. C., demonstrated microscopically the presence of microfilariæ in the blood of a mulatto woman, a native of Charleston, who had never been outside the city except for five years spent at Augusta, Ga. She had chyluria. He also demonstrated microfilariæ in the blood of a second negro with chyluria; this patient had never been outside of Charleston and its immediate neighborhood.

De Saussure [2] in 1890 reported 22 cases of chyluria with microfilariæ in the blood in people born in Charleston; 9 were colored males, 6 were colored females, 5 were white males and 2 were white females.

Johnson [3] in 1915 reported the blood examination of 400 individuals in the city of Charleston, S. C., representing for the most part consecutive routine admissions to the Roper Hospital; 77 or 19.25 per cent of that number harbored microfilariæ.

Johnson's table showing the groups of individuals examined and the percentage of infection found in each group is here inserted:

	Number examined.	Number infected.	Per cent infected.
White females	50	14	28
White males	50	9	18
Colored females	150	29	19.3
Colored males	150	25	16.7
	400	77	19.25

The writer in 1915 examined the 37 inmates of "The Old Folks' Home," a filarial focus in Charleston, and found that 13, or 35 per cent, of that number showed microfilariæ in their blood.

Columbia, S. C.

Blood examination was made in July, 1918, of 221 unselected individuals in two institutions at Columbia, S. C., and of this number three were found infected.

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[1] Guiteras, John: The *Filaria sanguinis hominis* in the United States. Medical News, 1886, v. 48, p. 399.

[2] De Saussure, P. G.: A clinical history of 22 cases of *Filaria sanguinis hominis*. Medical News, 1890, v. 56, p. 704.

[3] Johnson, F. B.: Filarial infection. An investigation of its prevalence in Charleston, S. C. Southern Med. J., v. 8, July, 1915, p. 630.

	White.		Colored.	
	Males.	Females.	Males.	Females.
State Hospital for the Insane	50	50	50	50
County poor house	4	5	8	4

Grand total, 221.

Data in regard to the three cases found infected are here inserted.

J. A., colored male, age 33, admitted to State Hospital February 22, 1918. Born in Charleston, 1882, and has always lived in Charleston. Has scars in right groin. Has no glandular enlargement and no elephantiasis. Urine passed was amber in color. Estimated 200 microfilariæ per c. c. of blood.

P. C., colored female, age 22, admitted to State Hospital July 13, 1918. Has always lived in Charleston. Came from Roper Hospital. Has no enlarged glands and no elephantiasis. Urine passed was amber in color.' Estimated 20 microfilariæ per c. c. of blood.

J. O. L., white male, age about 40. Admitted to State Hospital July 13, 1918, from Roper Hospital in Charleston. Has spent the past 20 years in Charleston. Has an enlarged gland size of end of thumb in left groin; no other enlarged glands. No elephantiasis. Says that his urine has never been milky or white. Estimated 75 microfilariæ per c. c. of blood.

Beaufort, S. C.

Beaufort is an old coast town of South Carolina, having a population of about 2,500.

The bloods of 67 people were examined April 17 to 20, 1916, for microfilariæ, all of which were negative except one. Five were private cases, 7 were inmates of The Old Folks' Home, 11 were male Negroes in the jail, and 44 were Negro students between the ages of 8 and 20 at the Penn School on St. Helena Island, which lies about 5 miles off the coast of Beaufort.

The following notes were taken on the one positive case found in Beaufort:

D. R., Negro, female, age about 45 years, was born on St. Helena Island; when very young she moved to Beaufort and has never lived elsewhere except for 48 days which she spent in Charleston in 1889. One leg below the knee is larger than the other. Blood examination showed seven microfilariæ on a slide of three large drops. Her mother is about 70 years of age and lived in Charleston from the time she was 10 years of age until she was about 20; examination of the mother's blood was negative for microfilariæ.

Georgetown, S. C.

Georgetown is a very old town of about 5,530 population, located near the coast. The blood examinations of 45 individuals were negative for microfilariæ. Twenty-nine of this number were Negro males in the chain gang, 11 were inmates of the jail, and 5 were private patients.

Dr. Gaillard, who had practiced for 16 years in Georgetown and the county, never saw a case of chyluria, and saw only one case simulating elephantiasis; he examined the blood of the latter several times at night for microfilariæ, but with negative results.

Dr. Sparkman has practiced about 25 years at Georgetown; he said that he had seen only two cases of chyluria, and that both were at Glenn Springs, Spartanburg County, S. C. He said that both were Charlestonians.

I spoke with other physicians who had practiced for a long time in Georgetown—with Drs. Moorer, Sawyer, and Bell—none of whom had seen chyluria or elephantiasis in the town.

Savannah, Ga.

Blood examinations of 287 unselected individuals at Savannah, Ga., were made in 1915 and were found negative. Those examined comprised the patients in the following institutions:

	Males.	Females.
Georgia Infirmary (colored)	75	50
Charity Hospital (colored)	50	50
St. Joseph's Hospital (white)	5	
United States Marine Hospital (only colored patients examined)	13	
Abrams' Home for the Aged (white)		44
Total	143	144

Dr. V. H. Bassett, bacteriologist for the past eight years of the city department of health, Savannah, Ga., informed me that only one case had come to his notice in Savannah in which microfilariæ had been demonstrated in the blood; this case was a native of the West Indies.

Milledgeville, Ga.

Blood examinations of 100 inmates of the State Insane Asylum, Milledgeville, Ga., were made in 1915 and all were found negative; this number embraced 45 white females and 55 colored females. The patients of the institution are drawn from all parts of the State, so that, to a limited extent, the examinations afford an index of conditions in many localities.

Jacksonville, Fla.

Blood examination was made May 22 and 23, 1916, of 200 individuals in the following institutions:

Duval County Hospital (mixed)	66
St. Luke's Hospital (white)	20
Brewster Hospital (colored)	14
City prison farm (mixed)	100
Total	200

All were found negative except two colored males; these were met with at the city prison farm and both were natives of Charleston, S. C. A brief summary of these two cases is here given:

No. 113. Negro, male; age, 23; born in Charleston, S. C.; left Charleston at age of 16 years for Fernandina, Fla., and thence to Jacksonville; shows no evidence of filariasis other than the blood findings: estimated 40 microfilariæ per c. c. of blood.

No. 140. Negro, male; age, 23; born in Charleston, S. C.; lived in Charleston until 18 years of age; came to Jacksonville two or three years ago; presents no evidence of filarial infection except the blood findings; estimated 360 microfilariæ per c. c. of blood.

While in Jacksonville I was told by Dr. H. Hanson, director of the laboratory of the State Board of Health at Jacksonville, that 20,000 blood smears had been examined for malaria in that laboratory coming from all over Florida, and that no microfilariæ had been found in them; malarial smears are usually taken in the daytime, however. He also said that not a single case of chyluria had come to their notice in five years in routine examination of urine in the laboratory at Jacksonville. In this connection the late Surg. R. H. von Ezdorf, United States Public Health Service, told me that in 13,500 thick blood smears taken in various Southern States and stained and examined for malaria no microfilariæ had been found.

Tampa, Fla.

Blood examination was made May 11 to 16, 1916, of 115 individuals at Tampa, Fla., in the following institutions:

	Males.	Females.
Hillsborough County Farm and Hospital (mixed)	64	15
County jail (colored)	31	
Private	3	2

Grand total, 115.

All examinations were negative for microfilariæ, except the following three:

No. 73 (private case). Spaniard, male; has spent many years in Tampa and Cuba. Microfilariæ were found on blood examination; this condition had, however, been diagnosed in 1911. Says he has intermittent chyluria.

No. 13. Spaniard, male; has spent the past 10 years in Tampa, while for 40 years previously he had lived in Habana. Says he has had chyluria. Microfilariæ in blood.

No. 21. German, male, born in Germany; came to New York during the Civil War and came to Tampa when the city was a "mere village;" spent six months in Charleston in 1868, and spent seven months in Charleston again some time later; he has never been in Cuba. Has an enlarged testicle. Microfilariæ were found on blood examination.

Examination of the annual reports of the Florida State Board of Health since 1907 showed only two cases in which the blood examinations had been positive. Both were reported by Dr. G. W. Simon, in charge of the State bacteriological laboratory at Tampa; one of these cases was reported in 1911 and probably was my case No. 73 above, while the other was reported in 1912 as simply positive for *Filaria bancrofti,* without any further details.

Mobile, Ala.

In Mobile, in July, 1918, blood examination of 131 unselected individuals distributed among the following institutions was negative:

	White.		Colored.	
	Males.	Females.	Males.	Females.
United States Marine Hospital	18		7	
City hospital	8	9	16	9
County poorhouse	17	21	16	10

Grand total, 131.

In addition, five private selected cases were examined for microfilariæ in the blood and found negative; two of these were white females with elephantiasis involving one leg below the knee; one was a white female who had had chyluria one year previously; one was a white male in whose blood microfilariæ were said to have been found in 1908 and who said that he had had attacks of chyluria; the other was said to have had chyluria.

New Orleans, La.

Blood examinations were made for microfilariæ in New Orleans in June, 1918, of 304 unselected individuals in the following institutions, all with negative results:

Institution.	White.		Colored.	
	Male.	Female.	Male.	Female.
Lafon Home, 1125 North Tonti Street			12	52
Faith Home, 3017 Bernadotte Street				11
Freedman's Home, Audobon Street			2	9
Lafon Home, South Robertson Street			4	12
German Protestant Home	21	23		
Touro Shakespeare Home	74	34		
Charity Hospital			50	
	95	57	68	84

Grand total, 304.

I was told while in New Orleans by Dr. C. C. Bass and by Dr. Wm. H. Harris that they had never seen microfilariæ in the fresh blood of the human subject in New Orleans, although special examinations had been made of those most likely to harbor the parasites, such as West Indians and Porto Ricans, and natives of New Orleans, including those who show an elephantiasis of the type on which Dr. Rudolph Matas[1] has written.

Table showing summary of surveys of 10 Southern cities for Microfilaria bancrofti.

Number of individuals examined.	Number of individuals found infected.	Estimated number of microfilariæ per c. c. of blood.
Charleston, S. C.:		
400[1]	77	....
Columbia, S. C.:		
221	3	200, 20, 75
Beaufort, S. C.:		
67	1	21
Georgetown, S. C.:		
45	....	....
Savannah, Ga.:		
287	....	....
Milledgeville, Ga.:		
100	....	....
Jacksonville, Fla.:		
200	2	40, 360
Tampa, Fla.:		
115	3	160, 130, 110
Mobile, Ala.:		
131	....	....
New Orleans, La.:		
304	....	....

[1] The figures given for Charleston are taken from Johnson's report because they comprise a very large number of individuals examined; the figures for the nine cities outside of Charleston are from my surveys.

An examination of the above table shows that of 400 individuals examined at Charleston, S. C., 77 were found to be infected with microfilariæ, whereas of 1,470 individuals examined in nine southern cities outside of Charleston only 9 individuals were found to harbor microfilariæ in their blood. A study of the table points to Charleston as a heavy focus of infection and indicates that Georgetown, S. C., Savannah, Ga., Milledgeville, Ga., Mobile and New Orleans are free from infection. The table also indicates that Columbia, S. C., Beaufort, S. C., Jacksonville, Fla., and Tampa, Fla., contain small foci of infection, inasmuch as 9 cases were found out of 603 individuals examined in those four cities. But the very interesting fact developed that these 9 cases found in Columbia, Beaufort, Jacksonville, and Tampa give histories either of having been born and raised in Charleston or of having spent a considerable period of time in Charleston or of having been born in Cuba which is a well-known filarial focus.

The data point to Charleston as the one active endemic focus of the disease in the South and indicate that cases found outside of Charleston have derived their infection either from residence in Charleston or from residence at some place outside of the United States, as in Cuba. The data indicate that the isolated cases found outside of Charleston do not constitute a new focus for transmission of the disease to people with whom they come in contact in their new locality. At first thought it is surprising that each case transplanted from Charleston to another city does not become a focus for the spread of the infection. This is accounted for on the grounds of the great difficulties which attend the mosquito transmission of the disease. Yellow fever, malaria, and filariasis are mosquito-borne diseases, and we know that both yellow fever and malaria do spread from one community to another in the South, but it has not been shown that filariasis has yet produced an endemic focus in a southern city outside of Charleston, although Charleston has been known to be an endemic focus of filariasis for the past 30 years and infected individuals have moved from Charleston to other cities and natives of other cities have returned home after spending considerable time in Charleston.

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[1] Matas, Rudolph: The surgical treatment of elephantiasis and elephantoid states dependent upon chronic obstruction of the lymphatic and venous channels. Am. J. Trop. Dis. & Prev. Med., v. 1 (1), July, 1913, p. 60, etc.

THE AGENCY OF THE MOSQUITO IN THE TRANSMISSION OF FILARIASIS.

We recognize the mosquito as the sole agent in the transmission of filariasis just as we recognize the mosquito as the sole agent in the transmission of yellow fever and malaria, but mosquito transmission is accomplished with much less degree of certainty and promptness in filariasis than in yellow fever and malaria, owing to certain marked essential differences in the process of mosquito transmission in the three diseases.

The *first* difference is that while in the case of malaria, and probably in yellow fever also, the parasite multiplies enormously in the mosquito, no multiplication of the filarial parasite whatever takes place in the mosquito; if the mosquito imbibes only one microfilaria from the blood of a filarial patient she can give back only one larva to the human subject.

The *second* difference in the process is that in filariasis every act or step of mosquito transmission is characterized by a small number of parasites; the number of microfilariæ imbibed by the mosquito is small and the number which successfully pass through the larval stage in her thoracic muscles and finally reach the proboscis to await the opportunity of an infecting bite is remarkably small; and the number of adults which reach a lymphatic gland of man is smaller still.

The *third* difference is that the filarial parasite as it leaves the mosquito's proboscis for man is of one sex or the other, and that multiplication must be deferred until a male and a female find lodgment within the *same* lymph gland of man and there grow and develop their genital organs preparatory to bringing about a fecundated adult female parent worm permanently located in that lymph gland and discharging her embryos into the blood stream. It is entirely possible that an individual might harbor a considerable number of adult parasites in his lymph glands and yet show no microfilariæ in his blood for the reason that those adults were so distributed among a number of lymph glands as to separate the sexes and thus preclude conjugation.

The *fourth* difference is found in the limited number of hours of maximum opportunity of infecting mosquitoes with filariæ; those hours being restricted to a few before midnight and a few after midnight.

The *fifth* difference in the process is that in the biting act which delivers the filarial larva back to man, the mosquito does not inject the larva into the blood stream of man but drops the larva on the free surface of the skin. The larva must then penetrate the intact skin and pass by the lymphatics to the nearest lymph gland.

Sixth. There are, moreover, certain additional difficulties attending the mosquito transmission of filariasis which weaken its efficiency. Probably half of the microfilariæ taken into a mosquito's stomach pass on into the hind gut and are lost instead of piercing the stomach wall and lodging in the thoracic muscles where the necessary larval changes must take place. Certainly a large proportion of the larvæ after they leave the thoracic muscles migrate backward into the abdomen and are lost, while others migrate into the legs or into the antennæ and palpi instead of into their necessary infecting position in the proboscis. The larvæ after liberation upon the skin are subject to being wiped off or washed off before they can penetrate the skin. The life of an infected mosquito is probably shortened by reason of harboring the worms.

REASONS FOR THE NONSPREAD OF FILARIASIS IN CITIES OUTSIDE OF CHARLESTON.

The nonspread of filariasis in cities to which infected Charlestonians have been transported is accounted for by the absence of mass blood infection in those transported cases and the absence of mass mosquito biting of those cases in their new localities.

Large numbers constitute an essential factor in the process of mosquito transmission of filariasis and malaria. In malaria the large number characterizes the parasites in the mosquito and in man and is brought about by multiplication; but in filariasis the number of parasites in the mosquito and in man is small, so that the large number essential to successful transmission must be found in the mosquitoes themselves—in mass mosquito biting. There is also an absence of large numbers of microfilariæ in the blood of cases of filariasis found outside of Charleston—an absence of mass blood infection. It is the combination of small blood infection and small mosquito biting that accounts for the nonspread of filariasis in cities, outside of Charleston. In the absence of mass blood infection and of mass mosquito biting, the transmission of filariasis is a rare accidental affair.

Microfilariæ are so large and their number is so small that they admit of being counted and therefore I will attempt to show by calculation the necessity of the two factors of mass blood infection and mass mosquito biting in bringing about a spread of the infection.

The nine cases of filariasis found in cities outside of Charleston (see table, page 16) showed an estimated average of only 124 microfilariæ per c. c. of blood. Supposing that an infected individual harbors 124 microfilariæ per c. c. of his blood and that a mosquito draws 1.15 milligrams (0.00115 c. c.) of blood at a bite, a mosquito would then have to bite such an infected individual seven times in order to get one microfilaria or if seven mosquitoes bit him, only one of the seven would imbibe a microfilaria. If this one microfilaria encountered no obstacles whatever in its cycle either in the mosquito's stomach, in the thoracic muscles of the mosquito or in its proboscis or on the human skin or in the lymphatics of man, it would successfully reach a lymph gland. Here its activities would, however, come to an end by reason of being a male or a female and by reason of the absence of one of the opposite sex.

The calculation goes to show the failure to convey filariasis of seven bites of mosquitoes which have fed upon a filarial patient whose blood content of microfilariæ is 124 per c. c.

A second set of 7 mosquito bites, if successful, might result in the deposit of a second filaria in some lymph gland of the body but quite distant from the first one. Mosquitoes have a wide choice of skin surface over which to distribute their bites and consequently there is a wide choice of lymph glands in which the infecting worm may lodge. It would not be difficult to distribute 10 infective mosquito bites over the body surface in such locations that no two of the ten implanted filariæ would meet in the same gland.

An individual harboring 10 adult filariæ implanted in 10 different lymph glands of his body and representing the bites of 70 mosquitoes which had bitten a patient harboring 124 microfilariæ per c. c. of blood, might not have a single microfilaria in his blood on account of the complete separation of the 10 adult males and females.

And even if a male and female should successfully lodge in the same lymph gland and if the female discharged 1,000 embryos into the blood stream and if the volume of the patient's blood was 6,000 c. c., the distribution of microfilariæ in the blood would be only one per 6 c. c. of blood; and if a *Culex fatigans* draws 1.15 milligrams of blood at a bite it would require 5,217 mosquito bites before a mosquito would succeed in imbibing one microfilaria.

WHY IS CHARLESTON AN ENDEMIC FOCUS OF FILARIASIS?

The questions as to when and from what place did Charleston derive her infection probably find their answer in that a shipload of slaves from a heavy focus of infection in Africa was landed in the city many year ago.

The answer to the question as to why the disease became endemic at that place is that the conditions of filarial transmission (mass blood infection and mass mosquito biting) found their fulfillment in the peculiarity of the condition that the infected individuals, being slaves, were held compactly together at that place freely exposed to mosquitoes.

The question as to why Charleston continues to remain an endemic focus of filariasis finds its answer in the fact of the continued presence in Charleston of a large number of cases of filariasis showing a high blood content of microfilariæ and exposed to constant biting by *Culex fatigans.*

These explanations are based upon the peculiarity of the process of mosquito transmission of filariasis that large numbers are essential. The various acts concerned in the process of mosquito transmission must be repeated in great numbers because of the small number of parasites concerned in each act of the process.

In order to bring about the transmission of filarial infection from one person to another, the infecting individual must harbor a large number of microfilariæ in his blood; he must be bitten by a large number of mosquitoes in order that some of those mosquitoes may become infected and a large number of infected mosquitoes must bite the individual who is to become infected.

METHOD OF EXAMINING BLOOD FOR MICROFILARIÆ.

In the method of examination employed, blood was collected between 10.30 p. m. and 1.30 a. m. by sticking, with a Hagedorn needle, the tip of the finger from which 6 to 8 large drops ($\frac{1}{4}$ to $\frac{1}{3}$ c. c.) of blood were squeezed out and allowed to fall on an ordinary clean glass slide. With a fresh toothpick the blood is evenly spread, before clotting, over the entire surface of the slide excepting a small area at one end reserved for a number scratched in with a diamond writing pencil. The thick blood films are dried in the air in a level position, protected from roaches and flies but freely exposed to the air. As soon as dry, the slides are stood upright, back to back, in Coplin jars containing preferably distilled water, or containing good tap water. The film should be dehemoglobinized as soon as dry in order to get a colorless film. An hour usually suffices for the water to entirely remove the hemoglobin from the red corpuscles, leaving the film colorless but otherwise unaltered. If desirable, the slides can be transferred to fresh water to complete the laking. As soon as laking of the hemoglobin is complete, the slides are examined while still wet on a mechanical stage under a 16-millimeter objective; in such preparations the glistening microfilariæ are readily distinguished. In routine work where a determination of the number of individuals infected is the sole object of the investigation, examination of the wet laked films is entirely satisfactory and was followed in our surveys.

It became necessary in some instances for the films to remain wet 48 hours while awaiting their turn for examination. In such cases, bacterial growth is likely to disintegrate the film and cause its detachment from the slide; a few drops of formalin added to the water will prevent such results.

STAINING OF MICROFILARIÆ.

Thick smears decolorized in distilled water as described above are sufficiently fixed to the slide by drying in the air.

Stain with Delafield's hemotoxylin for five minutes, steaming the stain over a flame as in staining tubercle bacilli. Wash thoroughly in tap water. Examination with the two-thirds objective shows that the preparation is overstained. Therefore, decolorize with acid alcohol sufficiently to differentiate with the low power the cells of the cell column of the parasite. Dehydrate in absolute alcohol, clear in oil of cloves or xylol, mount in Canada balsam. Staining of the parasites becomes necessary when it is desired to study their structure.

The polychrome stains commonly employed in blood examinations may also be used.

II. DETERMINATION OF THE MOSQUITO CARRIER AT CHARLESTON.

Conclusion as to which species of mosquito was acting as the mosquito host at Charleston was reached after making a mosquito survey in a heavily infected focus of filarial infection in Charleston (The Old Folks' Home) and after experimental demonstration of the infection in a certain species of mosquito found very prevalent in that focus.

A mosquito survey was made covering the determination of 1,000 mosquitoes collected at "The Old Folks' Home" in Charleston.

Experimental demonstration of the infection in the mosquito was derived by dissection of mosquitoes caught at the "Home" in bedrooms occupied by filarial patients and by dissection of mosquitoes which it had been attempted to infect experimentally in cages by allowing them to bite persons at the "Home" in whose blood microfilariæ were circulating.

A determination of 1,000 adult mosquitoes captured in rooms of "The Old Folks' Home" was made by the writer who classified them as follows:

Culex fatigans	902
Aedes calopus	91
Aedes solicitans	5
Anopheles quadrimaculatus	2
	1,000

In as much as the survey showed the prevalence of *Culex fatigans* and *Ædes calopus* almost to the exclusion of all other species, these two were selected for examination by dissection for evidence of filarial infection.

Culex fatigans and *Ædes calopus* represent by far the commonest house mosquitoes of the Southern States, and while these two species are constantly associated in that section, breeding together in house yards in the same water and in the same containers, *Ædes calopus* has never been proven an efficient host of *Filaria bancrofti.*

Culex fatigans, on the other hand, has long been proven a transmitter of *Filaria bancrofti,* and therefore it was to be expected that this species of mosquito would be bound to be the intermediary host at Charleston.

Determination as to whether a mosquito was infected with filariæ was reached by dissection of the mosquito and examination under the microscope of its thorax, abdomen, head, and proboscis for the presence of the developmental stages through which filariæ pass in the course of their migration through the organs of the mosquito host.

The mosquito dissections resulted in the finding of a high percentage of *Culex fatigans* infected with filariæ and the finding of all

Ædes calopus negative for that infection, thus showing conclusively that *Culex fatigans* is a transmitter of *Filaria bancrofti* at Charleston and that its constant associate, *Ædes calopus*, is incapable of such transmission.

INFECTIBILITY EXPERIMENTALLY OF CULEX FATIGANS WITH FILARIA BANCROTI.

Detailed data bearing upon the infectibility experimentally of *Culex fatigans* is found by reference to Tables Nos. 1 and 2.

Table No. 1 gives the data upon 27 *Culex fatigans* raised from larvæ in a noninfected community (Savannah, Ga.) and fed in cages at Charleston, S. C., upon patients heavily infected with *Filaria bancrofti;* 20 of the 27 mosquitoes so fed, or 74 per cent, contained larval forms of *Filaria bancrofti* at the time of dissection.

TABLE No. 1.—*Showing infectibility experimentally of Culex fatigans raised from larvæ in Savannah, Ga., and fed in cages at Charleston, S. C., upon blood of patients infected with Filaria bancrofti; 27 mosquitoes so fed were dissected at intervals, as shown below, 20 of which, or 74 per cent, contained larval forms of Filaria bancrofti.*

Dates of feeding of mosquitoes in cages upon filarial patients.	Dates of dissection of mosquitoes for filariæ.	Number of larvæ found in thorax on dissection.	Number of larvæ found in head and proboscis on dissection.	Number of larvæ found in abdomen on dissection.
Sept. 9, 10, 11, 13, 14, and 15, 1915. (See Table No. 5, column 1.)	Sept. 17, 1 dead	7 young larvæ	None	None.
	Sept. 17, 2 dead	None	None	None.
	Sept. 18, 1 dead	5 young larvæ	None	None.
	do	2 young larvæ	None	None.
	do	20 young larvæ	None	None.
	do	1 young larva	None	None.
	do	do		
	do	7 young larvæ		
	do	6 young larvæ		
	Sept. 18, 2 dead	None		
	Sept. 19, 1 dead	12 young larvæ		
	do	2 young larvæ		
	Sept. 19, 3 killed	None	None	None.
	Sept. 23, 1 dead	36 young larvæ; 1 half-grown, measured, stained, 0.47 by 0.027 mm.		None.
	Sept. 28, 1 dead	6 young larvæ, 1 mature larva.	None	1 mature larva measured fresh 1.175 by 0.022 mm.
	Sept. 30, 1 dead	1 young larva. 1 mature larva.		
	Oct. 3, 1 died	None	Head contained 3 mature larvæ; 1 measured fresh 1.48 by 0.025 mm. Proboscis contained 1 mature larva.	
	Oct. 11, 1 dead	None	Head contained 1 mature larva.	None.
	Oct. 12, 1 dead	1 entire mature larva	None	None.
	Oct. 12, 1 killed	Anterior and posterior ends of a mature larva seen.	1 motile mature larva seen in proboscis.	An anterior end of a mature larva seen.
	Oct. 18, 1 killed	1 larva measured fresh 0.68 by 0.020 mm. An anterior and a posterior end of a mature larva seen.	None	None.
	do	1 larva measured fresh 0.98 by 0.029 mm.	None	None.
	do	1 mature larva measured fresh 1.52 mm. in length.	1 mature larva	None.

Table No. 2 shows the infectibility experimentally of *Culex fatigans* caught at "The Old Folks' Home" in rooms occupied by nonfilarial patients but infected in cages by being allowed to feed upon the hands of filarial patients; 52 mosquitoes so fed were dissected for evidence of filarial infection, 34 of which, or 65 per cent, were found to be infected with filariæ.

TABLE No. 2.—*Showing infectibility experimentally of Culex fatigans caught at "The Old Folks' Home," Charleston, S. C., in rooms occupied by nonfilarial patients and allowed to feed in cages upon the hands of patients infected with Filaria bancrofti; 52 mosquitoes so fed were dissected, as shown below, 34 of which, or 65 per cent, were found to be infected with filariæ.*

Dates of feeding of mosquitoes in cages upon filarial patients.	Date of dissection of mosquito for filariæ.	Number of larvæ found in thorax of mosquito on dissection.	Number of larvæ found in head and proboscis of mosquito on dissection.	Number of larvæ found in abdomen of mosquito on dissection.
Sept. 16, 17, 18, 19, 20, and 21, 1915. (See Table No. 5, column 3.)	Sept. 18, 2 dead	None	None	None.
	Sept. 19, 1 dead	1 young larva		None.
	do	None	None	None.
	Sept. 20, 1 killed 9 hours after feeding.	1 microfilaria		None in stained blood.
	Sept. 21, 1 killed 9 hours after feeding.	6 microfilariæ, stained.		4 microfilariæ in stained blood.
	Sept. 23, 1 killed 36 hours after feeding.	1 microfilaria, stained		1 microfilaria, stained.
	Sept. 25, 1 killed 60 hours after feeding.	do	None	Do.
	Sept. 26, 1 died	1 mature larva, stained; 12 young larvæ.	None	None.
	Sept. 27, 1 died	2 young larvæ	None	None.
	Sept. 28, 1 dead	Several young larvæ.	None	None.
	do	2 young larvæ	None	None.
	Oct. 3, 1 dead	2 anterior ends of mature larvæ.	None	None.
	do	1 anterior end of mature larva.	None	None.
	Oct. 3, 2 dead	None	None	None.
	Oct. 4, 1 dead	22 young larvæ	None	
	Oct. 5, 1 dead	2 mature larvæ	1 mature larva in head.	2 mature larvæ.
	do	do		
	do	Piece of mature larva	2 mature larvæ in proboscis, stained.	Piece of mature larva.
	Oct. 7, 1 killed	None	None	None.
	Oct. 8, 1 killed	Pieces of mature larva.	None	None.
	do			Pieces of mature larva.
	do		1 mature larva in proboscis measured fresh 1.54 by 0.028 mm. 1 mature larva in head.	
	Oct. 8, 2 killed	None	None	None.
	Oct. 9, 1 dead		1 mature larva in proboscis. 1 mature larva in antenna.	Anterior portion of mature larva.
	Oct. 10, 1 dead	2 mature larvæ		
	Oct. 11, 1 dead	None	Posterior end of mature larva.	None.
	do	None	1 mature larva, measured fresh 1.44 by 0.025 mm.	
	do	None	1 mature larva	None.
	do	None	None	None.
	Oct. 12, 1 dead	Anterior end of mature larva.	None	Anterior end of mature larva.

TABLE NO. 2.—*Showing infectibility experimentally of Culex fatigans caught at "The Old Folks' Home," Charleston, S. C., in rooms occupied by nonfilarial patients and allowed to feed in cages upon the hands of patients infected with Filaria bancrofti; 52 mosquitoes so fed were dissected, as shown below, 34 of which, or 65 per cent, were found to be infected with filariæ*—Continued.

Dates of feeding of mosquitoes in cages upon filarial patients.	Date of dissection of mosquito for filariæ.	Number of larvæ found in thorax of mosquito on dissection.	Number of larvæ found in head and proboscis of mosquito on dissection.	Number of larvæ found in abdomen of mosquito on dissection.
Sept. 16, 17, 18, 19, 20, and 21, 1915. (See Table No. 5, column 3.)	Oct. 12, 1 dead	None	1 mature larva in an antenna, measured fresh 1.30 by 0.025 mm.	
	do	None	None	Piece of mature larva.
	Oct. 14, 1 killed		1 mature larva in proboscis. 1 mature larva in head.	
	do	None	None	Anterior end of mature larva.
	Oct. 14, 2 killed	None	None	None.
	Oct. 15, 1 killed	None	Anterior portion of larva in palpus.	Anterior and posterior ends of mature larva.
	do	None	1 mature larva in head.	
	do	Half-grown larva, measured fresh 0.76 by 0.033 mm.	None	1 mature larva.
	Oct. 15, 3 killed	None	None	None.
	Oct. 16, 1 killed	Piece of mature larva	None	None.
	do	None	2 mature larvæ in head, measured fresh 1.59 by 0.027 mm. and 1.45 by 0.027 mm.	None.
	Oct. 16, 1 dead	None	None	None.
	Oct. 18, 1 dead	Posterior end of mature larva.	1 mature larva in an antenna.	None.
	Oct. 19, 3 killed	None	None	None.

NOTE.—The possibility exists that some of the mosquitoes found infected in the above experiment derived their infection in nature before confinement to cages; i. e., that they were already infected before being allowed to feed in cages upon the hands of infected patients. This possibility arises from the fact of their having been caught in a filarial focus, "The Old Folks' Home," but in rooms occupied by nonfilarial patients. The percentage which may have thus derived their infection in nature can be estimated from a control series of dissections which was made of 100 *Culex fatigans* caught at "The Old Folks' Home" in rooms occupied by nonfilarial patients; 5 of that number or 5 per cent were found infected.

INFECTIBILITY IN NATURE OF CULEX FATIGANS WITH FILARIA BANCROFTI.

Detailed data bearing upon the infectibility in nature of *Culex fatigans* with *Filaria bancrofti* is found in Table No. 3. This table shows that of 65 *Culex fatigans* caught at "The Old Folks' Home" on the walls of rooms occupied by filarial patients, 13, or 20 per cent, were found on dissection to contain larval filariæ.

TABLE NO. 3.—*Showing infectibility, in nature, of Culex fatigans with Filaria bancrofti. Sixty-five Culex fatigans were caught Sept. 24 and Sept. 25, 1915, in Charleston at "The Old Folks' Home" on the walls of rooms occupied by filarial patients. These were dissected, as shown below, 13 of which, or 20 per cent, were found to contain larval filariæ.*

Dates of dissection of mosquitoes for filariæ.	Number of larvæ found in thorax of mosquito.	Number of larvæ found in head and proboscis of mosquito.	Number of larvæ found in abdomen of mosquito.
Sept. 26, 1 dead	1 young larva		
Sept. 26, 3 dead	None		
Sept. 27, 7 dead	None		
Sept. 28, 1 dead	2 larvæ, stained, length 0.87 mm. and 1.0 mm. 3 young larvæ.		
Sept. 28, 3 dead	None		
Sept. 29, 1 dead	1 young larva	None	Anterior end of mature larva.
Do	do	None	None.
Sept. 29, 6 dead	None	None	None.
Sept. 30, 1 dead	2 young larvæ, 1 half-grown larva.	1 mature larva seen emerging from end of proboscis.	None.
Do	1 crumpled larva	None	None.
Do	4 young larvæ	None	
Do		Proboscis contained 2 mature larvæ.	
Sept. 30, 5 dead	None	None	None.
Oct. 1, 1 dead	1 mature larva, 1 young larva.	None	None.
Do	24 young larvæ	None	None.
Do	1 mature larva, stained	None	
Oct. 1, 3 dead	None	None	None.
Oct. 2, 3 dead	None	None	None.
Oct. 3, 1 dead	2 young larvæ	None	None.
Oct. 3, 2 dead	None	None	None.
Oct. 5, 3 dead	None	None	None.
Oct. 6, 3 dead	None	None	None.
Oct. 7, 3 dead	None	None	None.
Oct. 8, 8 killed	None	None	None.
Oct. 12, 1 dead	None	1 mature larva in proboscis.	None.
Oct. 12, 3 dead	None	None	None.

NOTE.—Fifty *Aedes calopus* were caught Sept. 24 and 25, 1915, in Charleston at "The Old Folks' Home" on the walls of rooms occupied by filarial patients. All were dissected in saline solution, none of which contained larval filariæ, as shown below:

Dates of dissection of 50 Aedes calopus for filariæ.	Number of larvæ found in thorax of mosquito.	Number of larvæ found in head and proboscis of mosquito.	Number of larvæ found in abdomen of mosquito.
Sept. 29, 1 dead	None	None	None.
Sept. 30, 5 dead	None	None	None.
Oct. 1, 9 dead	None	None	None.
Oct. 2, 1 dead	None	None	None.
Oct. 4, 1 dead	None	None	None.
Oct. 5, 1 dead	None	None	None.
Oct. 6, 1 dead	None	None	None.
Oct. 7, 1 dead	None	None	None.
Oct. 8, 8 killed	None	None	None.
Oct. 9, 8 killed	None	None	None.
Oct. 12, 8 killed	None	None	None.
Oct. 23, 6 killed	None	None	None.

"INFECTED" BUT NOT NECESSARILY INFECTIVE."

Tables 1, 2, and 3 have given the numbers of *Culex fatigans* which on dissection were found to be infected with *Filaria bancrofti;* the term "infected" is taken to include all mosquitoes which harbored developmental forms of filaria in any part of their bodies (in head, thorax, proboscis, antenna, palpus, or abdomen).

It is evident that the bite of an "infected" mosquito may not be "infective" at all.

The bite of an infected mosquito is infective for man only at the time that that mosquito harbors mature larvæ actually in its proboscis; adult larvæ located in the mosquito's abdomen, thorax, head, legs, antennæ, or palpi can not, while so located, render infective their host's bite.

Table No. 4 summarizes the locations in the bodies of 67 infected *Culex fatigans* in which mature larvæ, young larvæ, and microfilariæ were found on dissection.

Table No. 4.—*Summary of Tables 1, 2, and 3, showing the total number of microfilariæ, young larvæ, and adult larvæ, with their seats of distribution in the different parts of the bodies of 67 infected Culex fatigans, found on dissection.*

Part of the mosquito's body in which filariæ were found on dissection.	20 infected *Culex fatigans.* (See Table 1.) 34 infected *Culex fatigans.* (See Table 2.) 13 infected *Culex fatigans.* (See Table 3.) — 67		
	Number of mature larvæ found.	Number of young larvæ found.	Number of microfilariæ found.
Proboscis	11		
Antenna	3		
Palpus	1		
Head	14		
Thorax	26	195	9
Abdomen	13		6
Total mature larvæ	68		

Table No. 4 shows that 67 infected mosquitoes contained on dissection only 68 mature larvæ and of this number of larvæ only 11 were located in the proboscis, i. e., only 11 were at the time of dissection so located as to render infective the bites of the 9 mosquitoes which harbored them. At the time of dissection, 13 mature larvæ were located in the abdomens of 12 mosquitoes and were, of course, entirely outside the possibility of rendering infective their host's bite. Three mature larvæ were headed into antennæ and 1 into a palpus. Fourteen mature larvæ were located in the mosquito's head and probably would reach the proboscis. Twenty-six mature larvæ were still in the thorax, with about equal chances of migrating either forward toward the proboscis or backward into the abdomen.

NONINFECTIBILITY OF ÆDES CALOPUS WITH FILARIA BANCROFTI.

Ædes calopus has never been proven an efficient host of *Filaria bancrofti*. *Microfilariæ bancrofti* taken into this mosquito's stomach do pierce the stomach wall in limited numbers and lodge in the thoracic muscles, but there they die as microfilariæ without development into the larval stage.

While our experimental studies of the infectibility of *Culex fatigans* were being made, it was thought advisable also to carry on a parallel set of experiments to test the infectibility of *Ædes calopus* with the species of filaria prevailing at Charleston, inasmuch as no experimental study has been reported having for its object the determination of the mosquito carrier of filariasis in the United States.

All experiments made and reported in Tables 1 and 3 to prove the infectibility of *Culex fatigans* were accompanied by an exactly parallel series of experiments to test the infectibility of *Ædes calopus*.

One hundred and twenty-four *Ædes calopus* (see Table 5, column 2) exposed to infection with *Filaria bancrofti* under exactly the same conditions as were 20 *Culex fatigans* found infected in Table 1, were all found negative for that infection on dissection. These 124 *Ædes calopus* were raised from larvæ in Savannah and were fed in cages in Charleston, between September 9 and 16, 1915, upon the blood of filarial patients, and were thereafter kept at a temperature of 26° C. and fed on simple sirup. They were dissected at times between September 19 and October 23 for evidence of filarial infection, but gave entirely negative results except for the first 6 which showed in the thoracic muscles from 1 to 4 unsheathed microfilariæ. (See Table No. 5, column 2.)

Fifty *Ædes calopus* (see Table No. 3, note), exposed to infection with *Filaria bancrofti* under exactly the same conditions as were 13 *Culex fatigans* found infected in Table No. 3, were all found negative for that infection on dissection. These 50 *Ædes calopus* were caught September 24 and 25, 1915, in Charleston at "The Old Folks' Home" on the walls of rooms occupied by filarial patients and were thereafter kept at a temperature of 26° C. and fed on simple sirup. They were dissected at times between September 29 and October 23 without finding in them any filariæ whatever. (See Table No. 3 note.)

TABLE No. 5.—*Showing method of feeding by which Culex fatigans was experimentally infected and Ædes calopus failed of infection with Filaria bancrofti at Charleston, S. C.*

Filarial patients.	Number of microfilariæ per c. c. of patient's blood examined at midnight.	Number of microfilariæ per c. c. of patient's blood examined at noon.	1915	Column 1. 400 *Culex fatigans*, raised from larvæ in Savannah, were placed, 100 in each of cages 1, 2, 3, and 4, into which filarial patients in Charleston introduced their hands, as indicated below.	Column 2. 400 *Ædes calopus*, raised from larvæ in Savannah, were placed, 100 in each of cages 1, 2, 3, and 4, into which filarial patients in Charleston introduced their hands, as indicated below.
Case 1	460	0	Sept. 9	Case 1 fed cages 2 and 3.	Cage 1 fed cage 4.
Case 2	2,000	21			Case 2 fed cage 1.
Case 3	3,000	14			Case 3 fed cages 2 and 3.
Case 4	2,465	26			
			Sept. 10	Case 2 fed cage 1. Case 3 fed cage 4.	Case 2 fed cage 2. Case 3 fed cage 1. Case 1 fed cage 3.
			Sept. 11	Case 1 fed cage 2. Case 2 fed cage 3. Case 3 fed cage 1.	Case 1 fed cages 1 and 2. Case 2 fed cage 4. Case 3 fed cage 3.
			Sept. 13	Case 2 fed cage 4. Case 3 fed cage 3.	Case 2 fed cage 3. Case 3 fed cage 4.
			Sept. 14	Case 4 fed cages 1, 2, and 3, condensed. Case 3 fed cage 4.	Case 4 fed cage 2. Case 3 fed cages 1 and 2.
			Sept. 15	Case 3 fed cage 4.	Case 3 fed cages 1 and 2. Case 4 fed cages 3 and 4.
			Sept. 16	On Sept. 16 there remained in the 4 cages only 27 surviving *Culex fatigans*. These were kept thereafter at 26° C. and fed on sirup. On dissection from time to time 20, or 74 per cent, showed larval forms of filariæ (See Table No. 1.)	On Sept. 16 there remained in the 4 cages 160 surviving *Ædes calopus*, which were kept thereafter at 26° C. and fed on sirup; 124 of these were dissected from time to time, but none were found infected with developmental forms of filariæ.
			Sept. 16	Column 3. 400 *Culex fatigans* were caught in Charleston at "The Old Folks' Home" on the walls of rooms occupied by nonfilarial patients and placed, 100 in each of 4 cages unsupplied with food other than blood of filarial patients, who introduced their hands into the cages as indicated below. After each feeding, the engorged mosquitoes were removed and placed in a common cage supplied with simple sirup.	
			Sept. 16	Case 4 fed 11 mosquitoes.	
			Sept. 17	Case 2 fed 13 mosquitoes.	
			Sept. 18	Case 2 fed 7 mosquitoes.	
			Sept. 19	Cases 2 and 3 fed 10 mosquitoes.	
			Sept. 20	Case 4 fed 3 mosquitoes.	
			Sept. 21	Case 3 fed 8 mosquitoes.	
				Total ...52 engorged mosquitoes. These 52 *Culex fatigans* which fed on filarial patients were dissected from time to time; 65 per cent proved to be infected with filariae. (See Table No. 2.)	

DIFFERENT BITING HABITS OF CULEX FATIGANS AND ÆDES CALOPUS.

In attempting to infect *Culex fatigans* and *Ædes calopus* with filariæ, they were confined to small cages about 1 foot square, into which filarial patients introduced their hands through gauze sleeves between 11 p. m. and 1 a. m. For the period of a week no food was supplied other than the opportunity to bite filarial patients every night or every other night. (See Table No. 5.)

The two species manifested very different biting habits under the conditions imposed by the experiment. In the presence of electric light *Culex fatigans* would not bite at all, and bit very poorly even after exclusion of light from their cage by a piece of thick black cloth in which the cage was wrapped. *Ædes calopus*, however, under the same conditions, attacked the hands of the patient immediately and filled with blood irrespective of light or darkness.

The reduction in numbers from 400 *Culex fatigans* to 27 in a week's time, when life depended upon blood-sucking at midnight in a small cage, indicates the almost absolute aversion which this species has to biting when confined to a small cage. There was no such reduction in numbers of *Ædes calopus*, since 160 of the original 400 survived at the end of the week; all except 43 of the 400 *Ædes calopus* filled with blood the first night.

III. PREVENTION OF SPREAD OF FILARIASIS.

Filariasis is entirely preventable, due to the fact of its transmission to man solely through the bites of mosquitoes which have become infected by biting infected individuals. Prevention of the spread of filariasis could be brought about in a community either by ridding the human host of microfilariæ through the use of some drug in the way that cinchonization aims to eradicate malaria from the circulation, or by ridding the community of the mosquito host of the filaria. Inasmuch as no means is known of reducing the number of microfilariæ in the blood, preventive measures are necessarily dependent upon mosquito eradication; this in the case of *Culex fatigans* is a comparatively easy undertaking, because *Culex fatigans* is a domestic mosquito, breeding in fresh water in back yards, in cisterns, rain barrels, tin cans, and other miscellaneous unnecessary artificial containers of rain water on the premises.

A compelling municipal ordinance or concerted action by every property owner directed to the abolishment of all collectors of water on the premises would practically rid the community of the *Culex fatigans* mosquito. If the municipality imposes upon the citizen the obligation to destroy all collectors of water on his premises, the municipality thereby obligates itself to furnish the citizen with the necessary means of getting good water; this the city can best do through the agency of a city-owned water system. A municipal water supply piped to every street, alley, and home in the town, coupled with a compelling municipal ordinance requiring the use of the city water exclusively and requiring the destruction of all collectors of rain water, will rid a community of filariasis by reducing the number of *Culex fatigans* to a negligible plane.

IV. ANATOMY OF THE PROBOSCIS IN RELATION TO FILARIA TRANSMISSION.

In order to understand the function of the proboscis in transmitting larval filariæ to man, one must know something of the anatomy of the proboscis and the mechanism of its action.

A casual glance at a mosquito's proboscis at rest gives one the impression of a simple scale-covered tube projecting from the mosquito's head (Pl. I, fig. 1); but if the mosquito is observed in the act of biting, the proboscis is very plainly seen to resolve itself into two parts, namely, an inclosed stilette bundle and an inclosing sheath. (Pl. I, figs. 2 and 3.)

If one will watch a mosquito undisturbed in the act of biting upon the back of his hand he will see with the naked eye that the proboscis resolves itself into an inclosed slender rigid piercing stilette bundle which penetrates the skin and acts as the conveyor of blood to the mosquito and an inclosing stout flexible sheath, slit along its dorsal border to enable it to bend or elbow away from the stilette bundle, leaving the latter free and unsheathed except where the distal end of the sheath surrounds the stilette bundle.

We have then to consider the anatomy of the stilette bundle and the anatomy of the proboscis sheath.

The stilette bundle is constructed for piercing the skin and for blood sucking and is made up of a labrum, two mandibles, two maxillæ and a hypopharynx. (Pl. II, figs. 1, 2, and 3.) The labrum is a long narrow grooved stiff spine ending in a penlike point; the mandibles and maxillæ are slender lancets apparently serrated at the ends; the hypopharynx is a flat plate carrying a small central canal throughout its length, which is continuous with the common salivary duct down which pass the malarial and yellow fever infection. The six parts of the stilette bundle are held together anatomically only at their proximal ends where they arise from the mosquito's head; throughout their length they are separate from each other except that they lie in apposition. The apposition of the flat hypopharynx to the groove of the labrum converts that groove into a canal and it is up this canal that blood passes to the mosquito's mouth by force of suction. When the mosquito bites, the entire stilette bundle is forced into the wound.

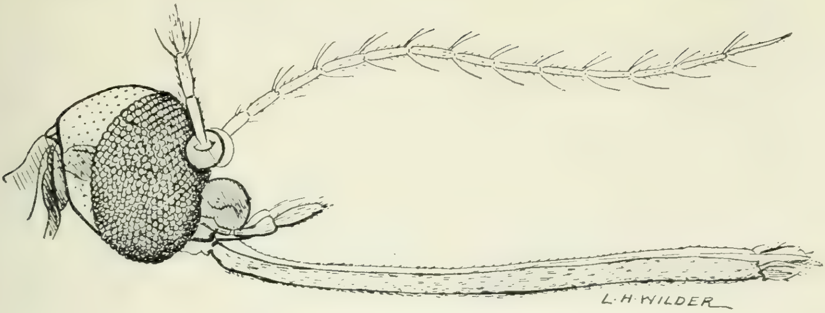

Fig. 1.—Proboscis of mosquito, grooved on its dorsal border and with the stilette bundle resting in the groove.

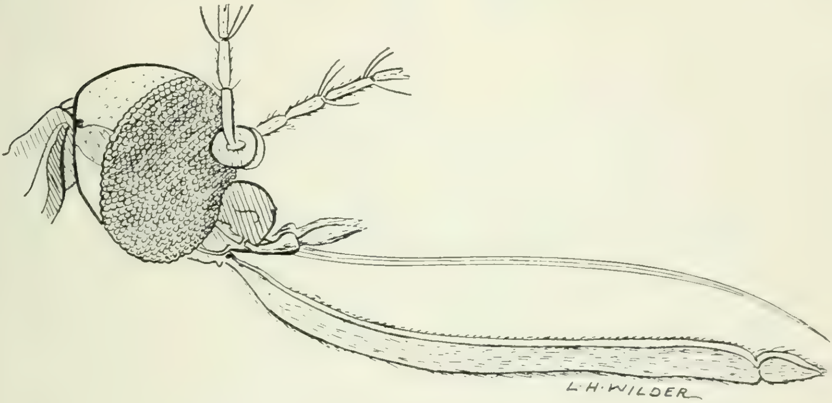

Fig. 2.—Same, but with stilette bundle entirely free.

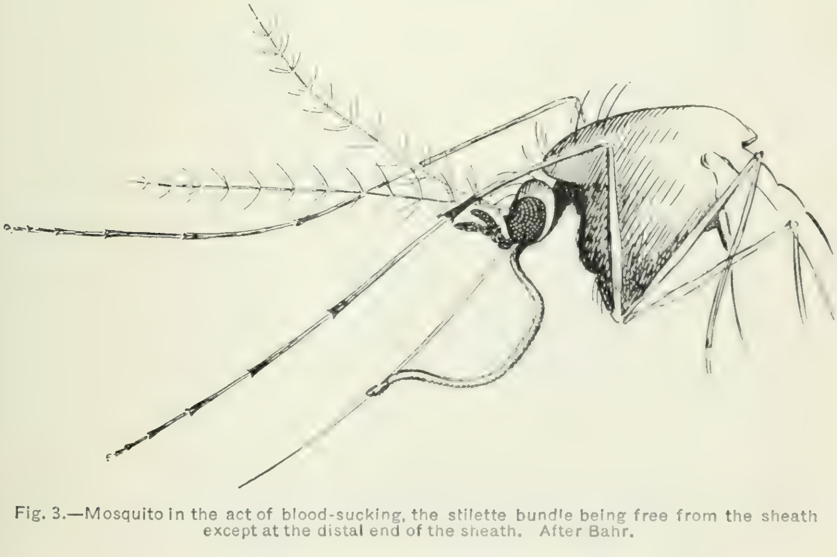

Fig. 3.—Mosquito in the act of blood-sucking, the stilette bundle being free from the sheath except at the distal end of the sheath. After Bahr.

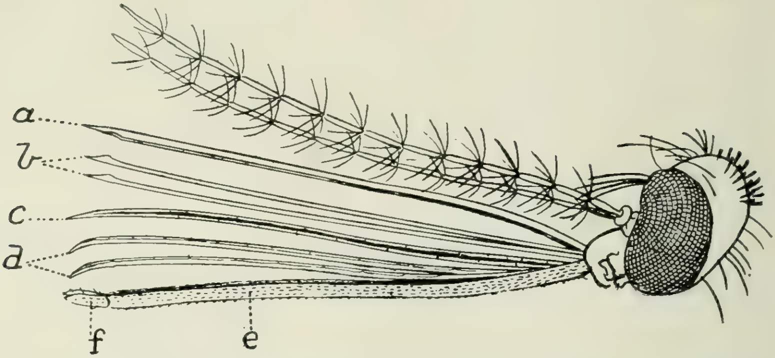

Fig. 1.—Proboscis (after Daniels) resolved into stilette bundle (a, labrum; b, two mandibles; c, hypopharynx; d, two maxillae); and sheath (e, labium).

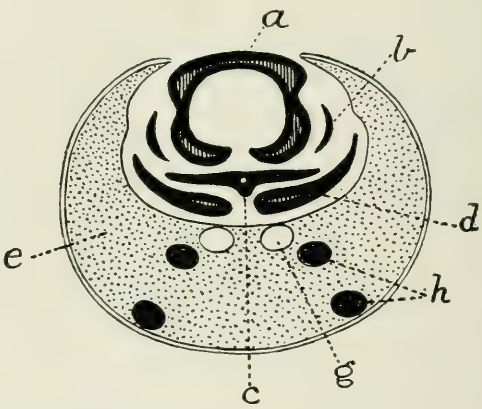

Fig. 2.—Transverse section of proboscis, after C. W. Daniels, laboratory studies in tropical medicine. Stilette bundle (a, labrum; b, two mandibles; c hypopharynx; d, two maxillae); Sheath (e, containing g, tracheae; h, muscles).

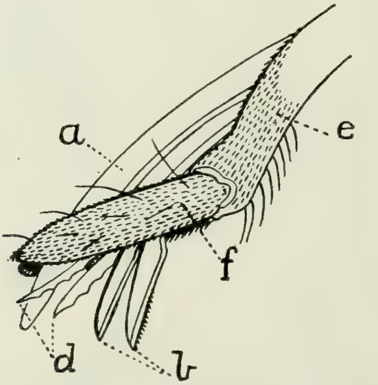

Fig. 3.—Anterior end of proboscis, showing the labellae (f) grasping the stilette bundle as in the act of piercing the skin; after Daniels, laboratory studies in tropical medicine.

The sheath is termed the labium. It is a cylindrical, stout, chitinous organ, covered with scales, and bearing a deep groove on its dorsal border in which lies the stilette bundle but from which the stilette bundle escapes in the act of blood sucking. It is composed of connective tissue in which lie a pair of tracheæ and also muscles and nerves. Its proximal end communicates with the head and its distal end terminates in two articulating lobes (labellæ) which during the biting act separate from each other and are applied to the surface of the skin and probably serve to grasp the stilette bundle and help to support the stylets as they sink between the two labellæ deeper and deeper into the skin while the labium is being bent down out of the way into a loop.

THE TWO COURSES OF FILARLÆ THROUGH THE PROBOSCIS.

The inward course and the outward course of filariæ through the proboscis are very different. Their inward course is by way of the stilette bundle which is intimately associated with blood sucking, but their outward course is by way of the proboscis sheath or labium which has no relation to blood sucking. Necessarily the inward course of microfiliariæ is with the ingested blood which is through the canal formed by the apposition of the hypopharynx to the groove of the labrum and thence to the mosquito's stomach. The microfilariæ after penetrating the stomach wall and undergoing metamorphosis in the thoracic muscles migrate as larvæ through the connective tissue spaces of the mosquito, first through its neck, then through its head and finally into the connective tissue spaces of the labium. The labium is a closed structure; it has no openings through which the larvæ can escape from their mosquito host.

If an infected mosquito is observed under the microscope, the filiariæ can be watched moving about in the connective tissue spaces of the labium awaiting an opportunity to get back into man at the time of the next blood sucking.

When the time of the infecting bite arrives, the stilette bundle penetrates the skin and plugs the opening; the labium bends or elbows away from the stilette bundle except where the labellæ grasp it; the filariæ escape from the inner thin surface of the labellæ by penetrating the intact thin Dutton's membrane; they fall on the skin close to the wound which the stilette bundle fills; they then penetrate the normal skin and pass to the lymphatics and lymph glands, but do not enter the blood stream through the mosquito puncture.

Under the headings "Filariasis defined," "The life cycle of *Filaria bancrofti*," "The anatomy of the mosquito's proboscis," and "The two courses of filariæ through the proboscis," no claim for originality is intended. Credit for the various discoveries which go to complete our knowledge on these subjects belongs to various authors and will be found on reference to the following publications:

ANNETT, Dutton & Elliott; Report on the malaria expedition to Nigeria. Pt. 2, Filariasis. Mem. 4 of the Liverpool School of Tropical Medicine. 1901.

BAHR: Filariasis and elephantiasis in Fiji. London, 1912.

DANIELS: Studies in laboratory work. London, 1903.

FÜLLEBORN: Die Filarien des Menschen. Handb. d. pathogenen Mikroorganismen, v. 8, 1913, pp. 185–344, figs. 1–41, pls. 1–6. (2. vermehrte Aufl.)

GILES: A handbook of the gnats or mosquitoes, giving the anatomy and life history of the Culicidae. 2. ed. London, 1902.

LEBREDO: Metamorphosis of Filaria in the body of the mosquito (*Culex pipiens*). J. Infect. Dis., Suppl. 1, 1905, p. 332.

LOW: A recent observation on *Filaria nocturna* in Culex, probable mode of infection of man. Brit. Med. J., 1900, v. 1, p. 1456–57.

MANSON: Tropical diseases, a manual of the diseases of warm climates. 5. ed. London, 1914.

THEOBALD: Monograph of the Culicidæ of the world. v. 4, 3. London, 1907.

HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

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PLATE III.

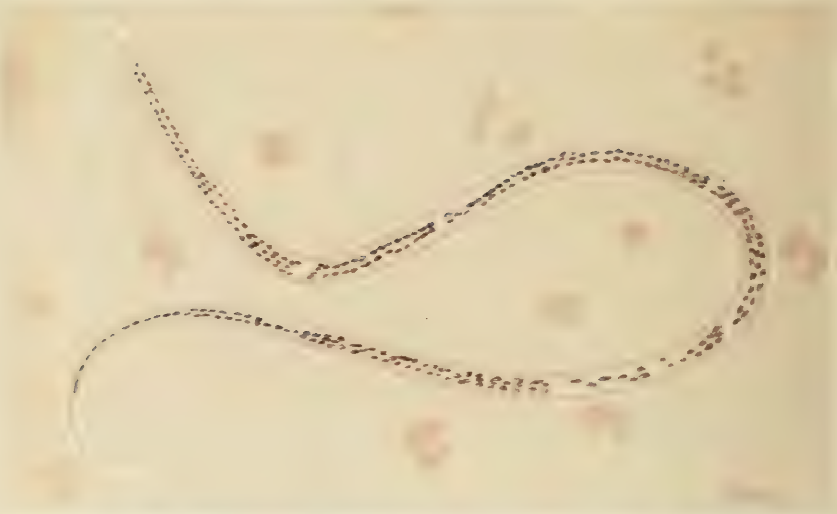

FIG. 1. *Microfilaria bancrofti* with sheath, from blood taken at Charleston, S. C. Stained with hematoxylin.

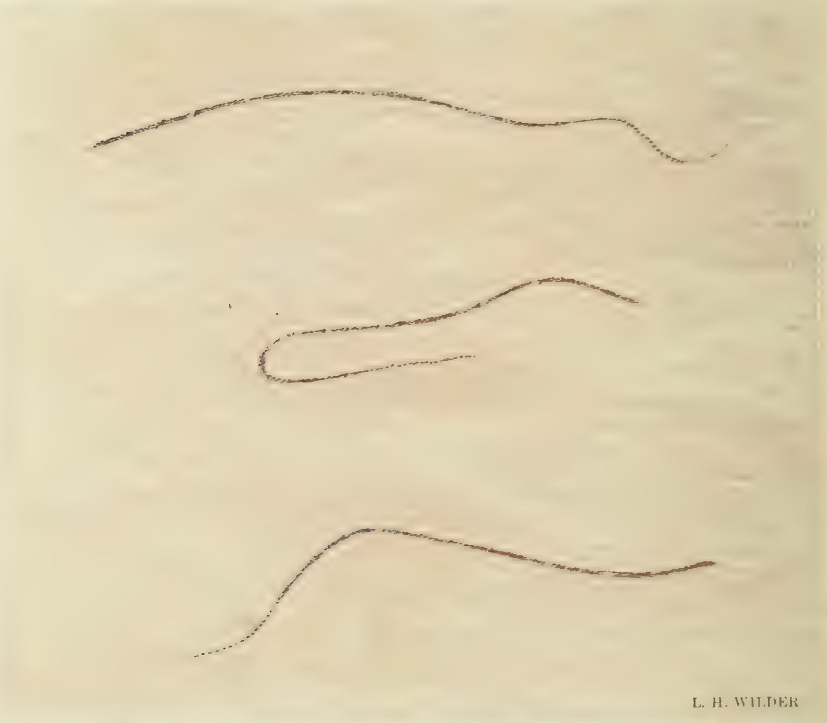

FIG. 2. Filariæ (without sheath) in thoracic muscles of *Culex fatigans*, 9 hours after ingestion of filarial blood; infected at Charleston, S. C. Teased preparation.

PLATE IV.

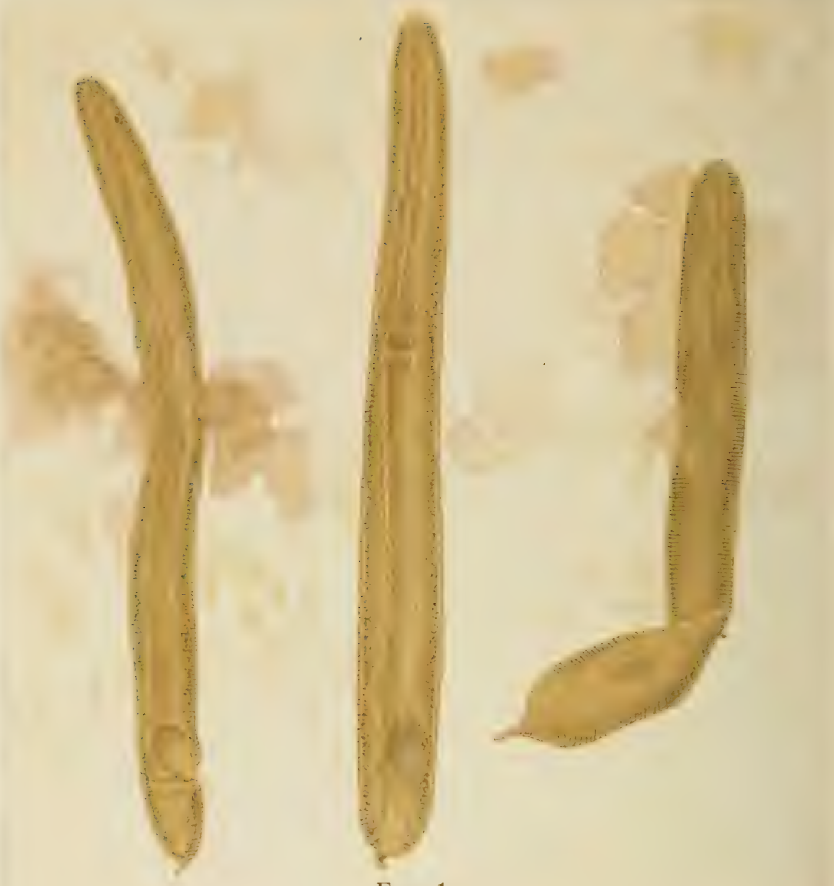

FIG. 1.

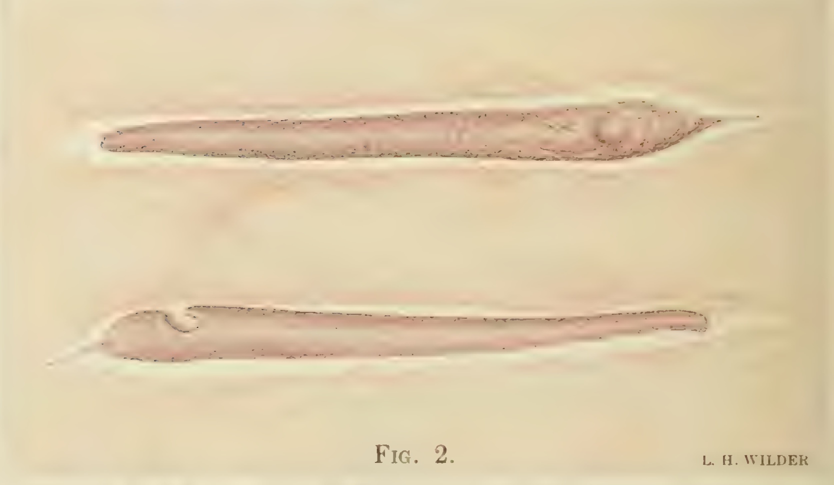

FIG. 2.

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FIGS. 1 and 2. Larvæ of *Filaria bancrofti* in thoracic muscles of *Culex fatigans* within 10 days after ingestion of blood; infected at Charleston, S. C. Teased preparations.

PLATE V.

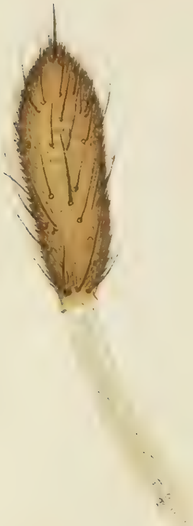

FIG. 1.

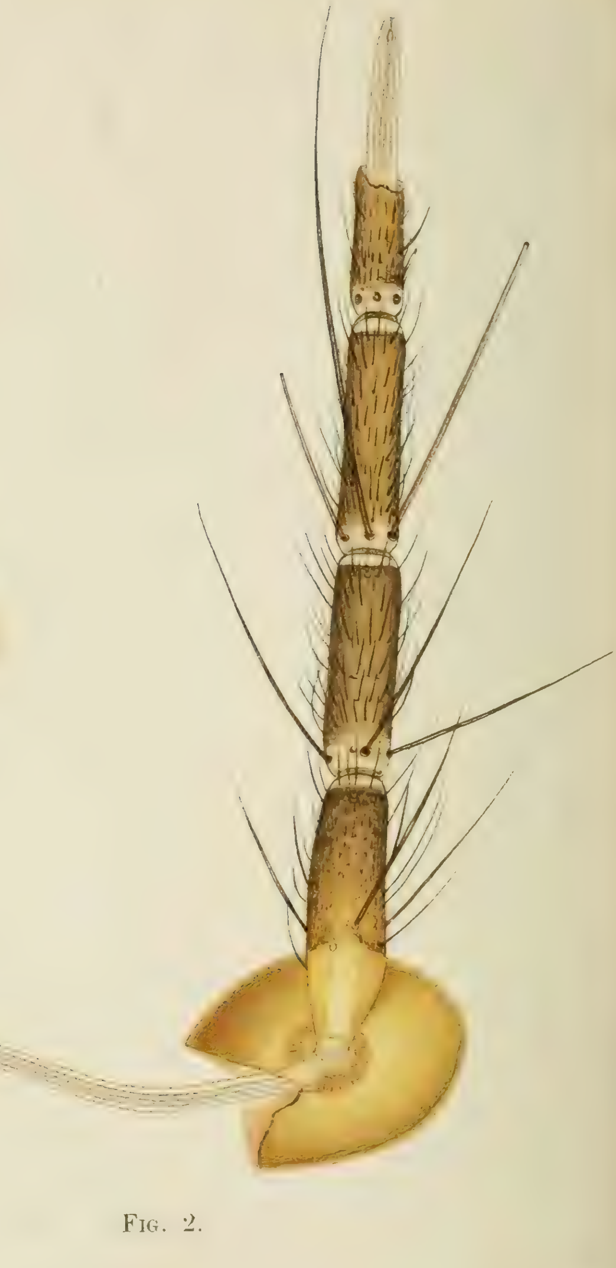

FIG. 2.

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FIG. 1. Larva headed into a palpus.

FIG. 2. Larva of *Filaria bancrofti* headed into an antenna of *Culex fatigans*. From mosquitoes infected at Charleston, S. C.

PLATE VI.

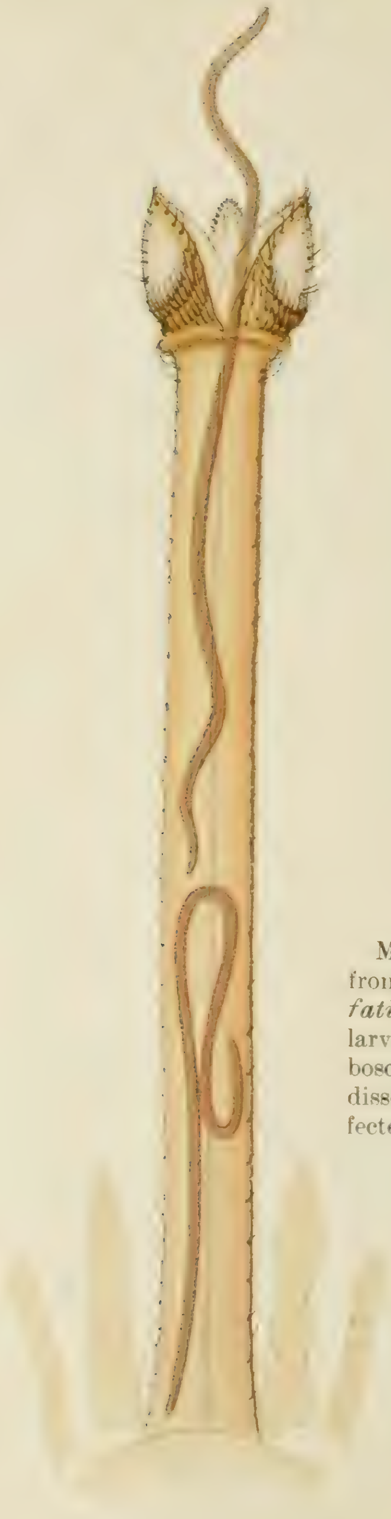

FIG. 1.

Mature larva escaping from proboscis of *Culex fatigans;* one mature larva coiled in base of proboscis. From mosquito dissected after being infected at Charleston, S. C.

[illegible]

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PLATE VII.

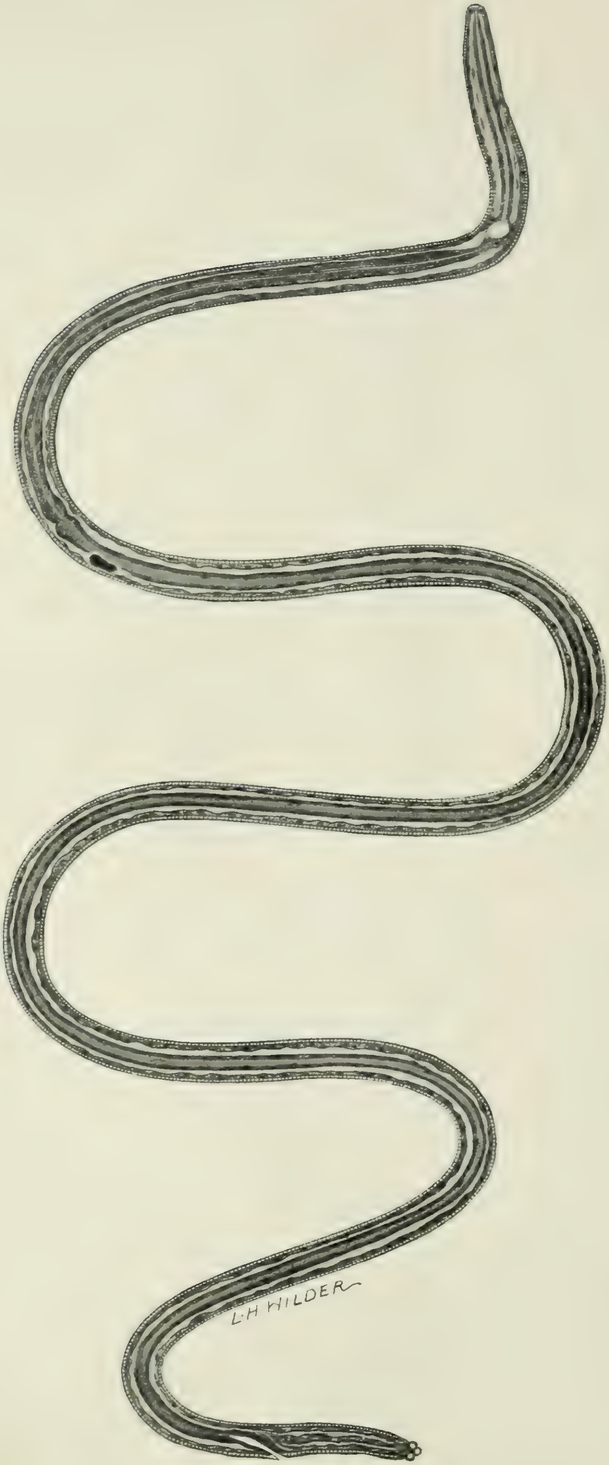
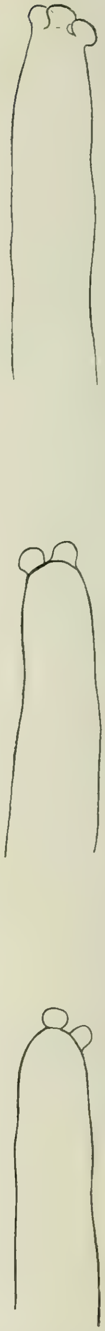
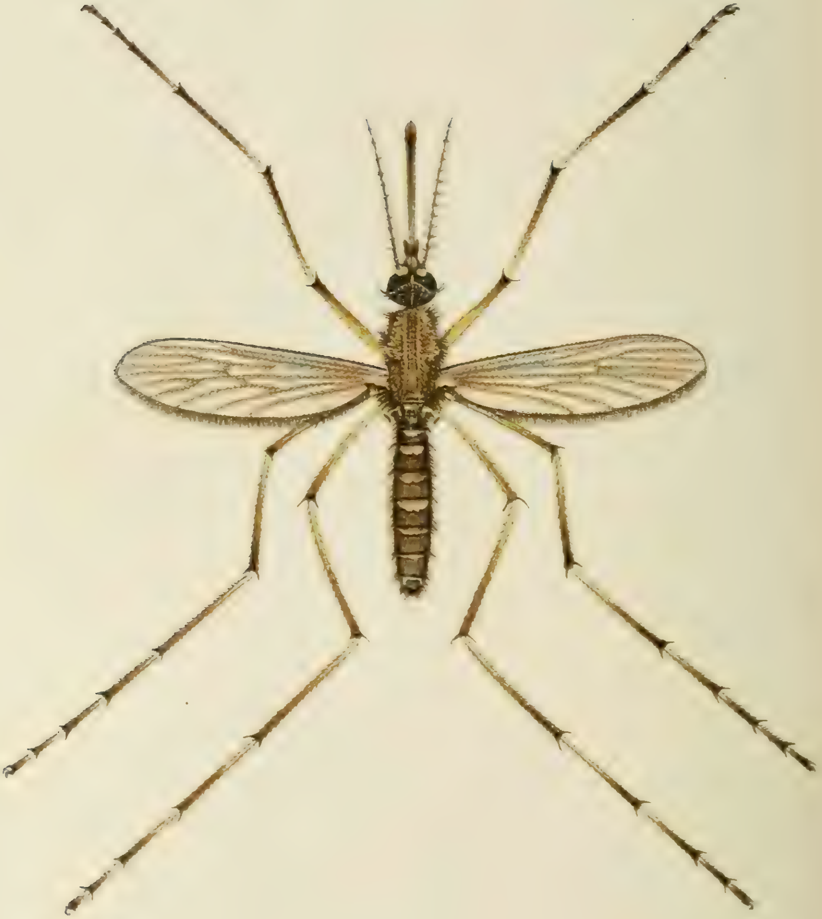


Fig. 1.—Larva of *Filaria bancrofti* at time of escape from proboscis of *Culex fatigans.*



Figs. 2, 3, and 4.—Showing two and three caudal papillae of larvae of *Filaria bancrofti* at time of escape from *Culex fatigans.* From preparations made on dissection of infected mosquitoes at Charleston, S. C.

PLATE VIII.



[illegible] BALTIMORE

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FIG. 1. Adult female *Culex fatigans.*

PLATE IX.

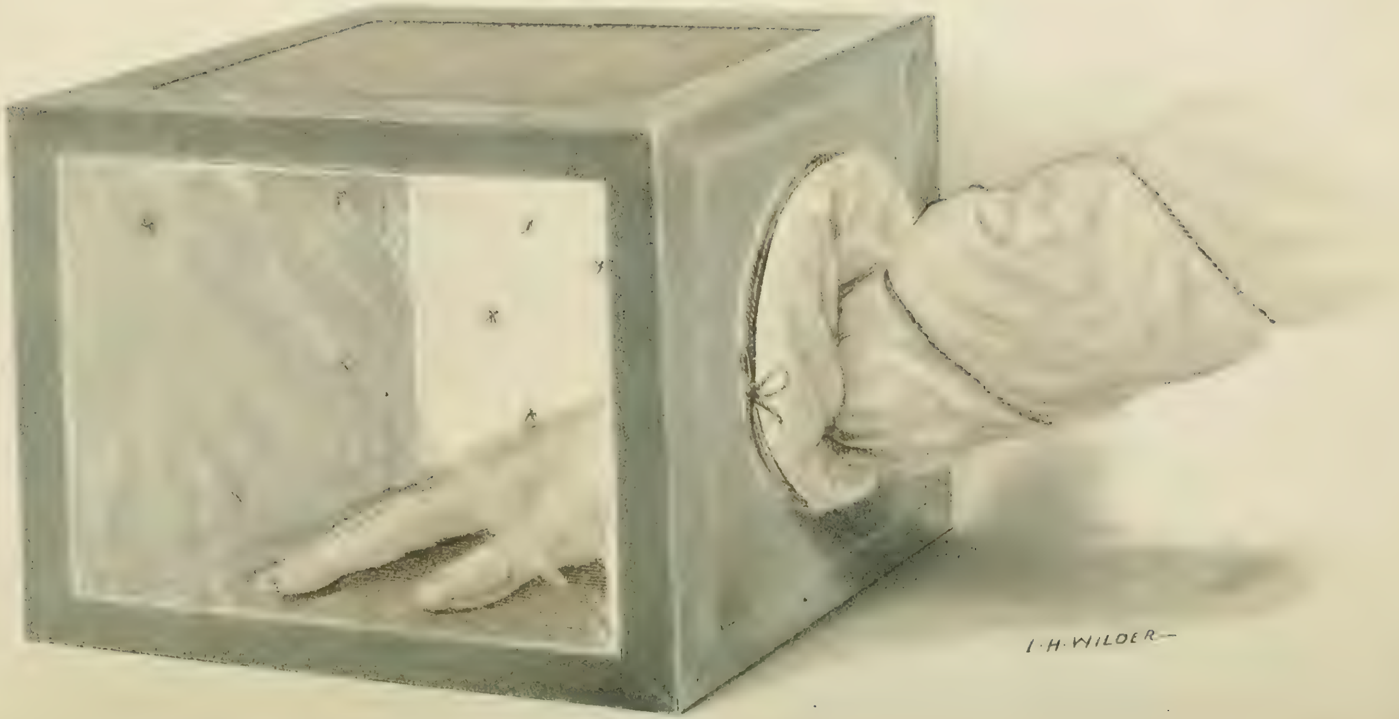

Fig. 1. Mosquito cage and sleeve.

PLATE X.

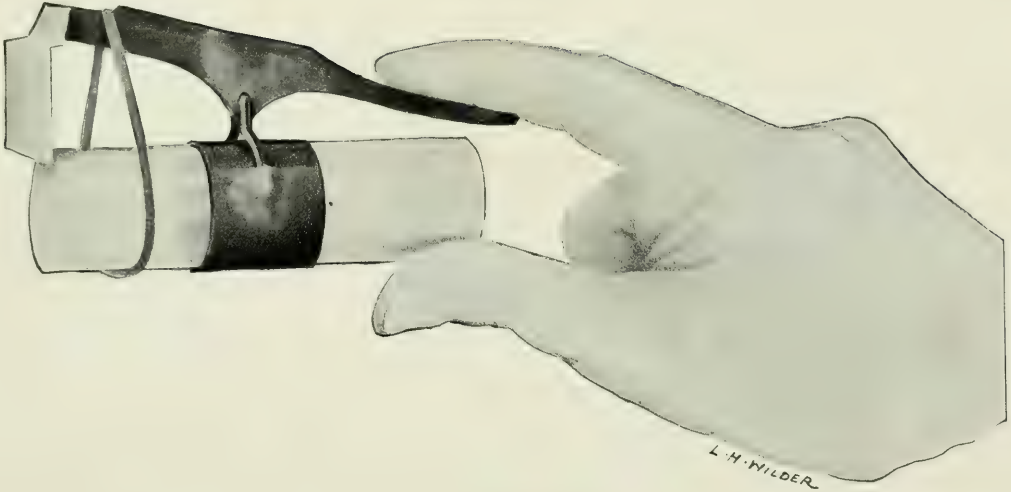

Fig. 1.—Mosquito catcher.

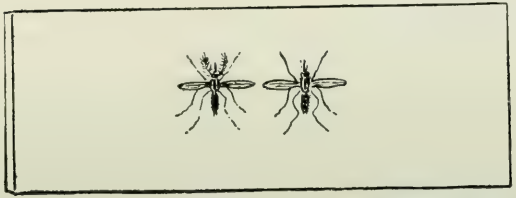

Fig. 2.—Method of mounting mosquitoes on glass slide with mucilage or Canada balsam.

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